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(54) Title: IDENTIFICATION OF BROADLY REACTIVE HLA RESTRICTED T CELL EPITOPES

(57) Abstract

This application discloses five new amino acid supermotifs and corresponding grouping of HLA molecules (*i.e.*, HLA supertypes that bind peptides that bear these supermotifs). Peptides epitopes bearing these supermotifs are selected for use in vaccines and other pharmaceutical compositions. The new supermotifs are used to screen known disease–related targets, as well as targets that will be determined in the future.

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IDENTIFICATION OF BROADLY REACTIVE HLA RESTRICTED T CELL EPITOPES

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Application 60/117,486, filed 1/27/99, which is incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

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BACKGROUND OF THE INVENTION

Over the past decade, an understanding of how T cells recognize antigen has emerged. A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601, 1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to particular gene-encoded HLA molecules have been identified and are described herein and are set forth in Tables 1, 2, and 3 (*see* also, *e.g.*, Southwood, *et al.*, *J. Immunol.* 160:3363, 1998; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995; Rammensee *et al.*, SYFPEITHI, access via web at : http://134.2.96.221/scripts.hlaserver.dll/home.htm; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al.*, *Cell* 74:929-937, 1993; Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; Sidney *et al.*, *J. Immunol.* 157:3480-3490, 1996; Sidney *et al.*,

Human Immunol. 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics*, in press, 1999).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g.*, Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al.*, *Immunity* 4:203, 1996; Fremont *et al.*, *Immunity* 8:305, 1998; Stern *et al.*, *Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA molecules.

In the past few years, evidence has demonstrated the value of a peptide epitope approach to disease treatment. More specifically, vaccination, with either dominant or subdominant epitopes has been shown to be protective against acute or chronic viral infection in experimental systems such as influenza or LCMV infection (Oukka, M. *et al.*, *J. Immunol.* 157:3039, 1996; Tourdot, S. *et al.*, *J. Immunol.* 159:2391, 1997; van der Most, R. G., *et al.*, *J. Immunol.* 157:5543, 1996; van der Most, R. G., *et al.*, *J. Virol.* 71:5110, 1997; An, L.-L., *et al.*, *J. Virol.* 71:2292, 1997). A variety of studies have also demonstrated the value of epitope-based vaccines in parasitic and microbial infections (Le, T. P., *et al.*, *Vaccine* 16:305, 1998; Wang, R., *et al.*, *J .Immunol.* 157:4061, 1996; Franke, E. D. *et al.*, *J. Immunol.* 159:3424, 1997), and cancer (Iwasaki, A., *et al.*, *Cancer Immunol. Immunother* .45:273, 1998; Melief, C. J. M., *et al.*, *Curr. Opin. Immunol.* 8: 651:1996; Mayordomo, J. I., *et al.*, *Nature Med.* 1:1297, 1995; Mandelboim, O. *et al.*, *Nature* 369:67, 1994; Lethe, B. *et al.*, *Eur. J. Immunol.* 22:2283, 1992; Van den Eynde, B., *et al.*, *Eur. J. Immunol.* 24:2740, 1994; Bloom, M. B., *et al.*, *J. Exp. Med.* 185:453, 1997; Morgan, D. J., *et al.*, *J. Immunol.* 160:643, 1998; Mayordomo, J. I., *et al.*, *J. Exp. Med.* 183:1357, 1996; Vierboom, M. P. M., *et al.*, *J. Exp. Med.* 186:695, 1997).

Additionally, studies have shown the clinical efficacy of epitope vaccines in the treatment of human neoplasias, such as melanoma. (Nestle, F. O., *et al.*, *Nature Med.* 4:328, 1998; Rosenberg, S. A., *et al.*, *Nature Med.* 4:321, 1998). In one case, tumor antigen epitopes were pulsed onto dendritic cells (Nestle, F. O., *et al.*, *Id.*) while in the other case, an epitope previously engineered for high HLA binding (Parkhurst, M. R., *et al.*, *J. Immunol.* 157:2539, 1996), was effective when simply delivered in a mineral oil emulsion (Rosenberg, S. A., *et al.*, *Nature Med.* 4:321, 1998).

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules encoded by myriad genetic alleles. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used that are specific for HLA molecules corresponding to each individual HLA allele. Impractically large numbers of epitopes would therefore have to be used in order to cover populations of any size; this is particularly true for ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA molecules for use in epitope-based vaccines. The greater the number of HLA molecules bound, the greater the breadth of population coverage by the vaccine.

The present invention fulfills this need. More specifically, this application discloses five new amino acid supermotifs and their respective corresponding groups of HLA molecules (*i.e.*, HLA supertypes) that bind peptides that bear these supermotifs. Epitopes comprising the supermotifs can bind multiple allele-specific HLA molecules. Accordingly, such supermotif-bearing peptide epitopes are selected for use in vaccines and other pharmaceutical compositions that can be targeted to a genetically diverse population. As appreciated by those in the art, the new supermotifs are used to screen known disease-related targets, as well as targets that will be determined in the future.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

SUMMARY OF THE INVENTION

Disclosed herein are several HLA supertypes and the corresponding amino acid supermotifs bound thereby. This invention furthers the ability to develop epitope-based vaccines that cover large sements of the population and prevent and/or treat neoplasias (such as cancer) and infectious diseases (such as AIDS, malaria, hepatitis B or C virus infection, and tuberculosis).

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. There is evidence that the immune response to whole antigens is directed largely toward variable regions of the antigen, allowing for immune escape due to mutations. The epitopes for inclusion in an epitope-based vaccine are selected from conserved regions of antigens of pathogenic organisms or tumor-associated antigens, which thereby reduces the likelihood of escape mutants. Furthermore, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines.

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (*e.g.*, both CTL and/or HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by entire infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen. Thus, patient-to-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from that pathogen in a vaccine composition.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, *e.g.*, so that peptides that are able to bind to multiple HLA antigens do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage,

and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

As disclosed herein, the broad applicability of the peptide epitope-based approach is a major strength of the invention. Accordingly, identification of supermotif-bearing epitopes from a variety of target pathogens is performed in accordance with the invention. Target pathogens fall, without limitation, into several categories:

1. Antigenic epitopes from viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human papilloma virus (HPV).

This category also includes large viruses which have so far not been systematically analyzed because of the large number of different proteins encoded by the virus. Examples of these are Herpes Simplex Virus 2, Epstein-Barr Virus, and Cytomegalovirus.

2. Antigenic epitopes from bacteria and parasites, such as *Plasmodium falciparum*. This category also includes epitope from bacteria and parasites that have recently been targeted in genome sequencing efforts. Examples include *Chlamydia trachomatous, Enterococcus fecalis, Trypanosoma pallidum* and *Helicobacter pylori*.

3. Antigenic epitopes from sources previously known and sequenced, but not previously identified as being associated with a malignancy, *i.e.*, tumor-associated antigens.

4. Antigenic epitopes from antigens newly identified as being associated with a malignancy.

5. Antigenic epitopes from known tumor-associated antigenic sources. The antigens include, but are not limited to, p53, carcinoembryonic antigen (CEA), the MAGE family of antigens, HER2/neu, and prostate cancer-associated antigens such as prostate specific antigen (PSA), prostate specific membrane antigen (PSM), prostatic acid phosphatase (PAP), and human kallikrein2 (hK2).

Also in accordance with the invention are computers and software that identify motif bearing subsequences in a natural or synthetic peptide of interest. Computers and software are also employed to generate analogs of a native protein

sequence. Typically, an analog is selected for use which has enhanced immunogenic and/or HLA cross-reactive binding properties.

An alternative modality for defining the superomotif-bearing peptides in accordance with the invention is to recite the physical properties, such as length; primary, secondary and/or tertiary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptides is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (e.g. pocket configuration and charge distribution) and reciting that the peptide fits and binds to said pocket or pockets.

It is also to be appreciated that supermotif-bearing peptide epitopes in accordance with the invention can exist as actual peptides, or as nucleic acids (DNA or RNA) that encode the peptides. A peptide of the invention can be polyepitopic in that it contains an epitope of the invention, along with one or more additional epitopes. Peptides identified in accordance with the invention can, therefore, comprise multiple epitopes in accordance with the invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, *e.g.*, Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

"Enhanced population coverage" means that a subject peptide is recognized by allele specific HLA molecules present in more than 55% of the general population.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably. It is to be appreciated, however, that isolated or purified protein or peptide molecules larger than and comprising an epitope of the invention are still within the bounds of the invention.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see*, *e.g.*, Stites, *et al.*, IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, CA, 1994).

An "HLA supertype or superfamily", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC_{50}'s." IC_{50} is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC_{50} values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents

used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50}, relative to the IC_{50} of a standard peptide.

Binding may also be determined using other assay systems including those using: live cells (*e.g.*, Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 19990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (*e.g.*, Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (*e.g.*, Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA systems (*e.g.*, Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (*e.g.*, Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or assembly (*e.g.*, Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC_{50}, or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 50 and about 500 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL response. Thus, immunogenic peptides of the invention are

capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response to the antigen from which the immunogenic peptide is derived.

The terms "interacting", "associating", "testing", and "contacting" are synonyms that refer to bringing a first moiety and a second moiety into proximity so as to effect binding or a biological response.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

"Obtaining" in the context of "obtaining" a nucleic acid is defined as providing a naturally occurring, recombinant, or synthetic peptide, or a nucleic acid encoding a peptide.

A "pathogen" may be, *e.g.*, an infectious agent or a tumor-associated molecule.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13

residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues.

"Pharmaceutically acceptable" refers to a non-toxic, inert, and/or physiologically compatible composition.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves of the HLA molecule. In one embodiment, for example, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position, *e.g.*, position 9, of a 9-residue peptide epitope in accordance with the invention. The carboxyl terminal position of an epitope may also be at other positions, *e.g.*, position 8, 9, 10, or 11. The epitope may be a component of a larger peptide fragment, wherein the C-terminal position of the epitope does not correspond to the C-terminal position of the peptide fragment. The primary anchor positions for each motif and supermotif are set forth in Table 1. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif. Analogs can also be created by altering residues at positions that are not anchors.

"Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA molecules.

"Synthetic peptide" refers to a peptide that is not naturally occurring, but is man-made using such methods as chemical synthesis or recombinant DNA technology.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single

letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. Symbols for the amino acids are shown below. In addition to these symbols, "B"in the single letter abbreviations used herein designates α-amino butyric acid.

Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

A. HLA Supertypes and Supermotifs. A conceptual view of MHC Polymorphism

1. Discovery of HLA Supertypes

The large degree of HLA polymorphism is a factor to be taken into account with the epitope-based approach, if epitopes capable of binding to a large number of different MHC molecules are idenified. To address this factor, epitope selection encompassing identification of peptides capable of binding multiple HLA molecules is preferably utilized.

Several amino acid supermotifs have been identified, each of which corresponds to the ability to bind several allele-specific HLA molecules. The allele-specific HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype." Early studies defined the motifs recognized by some of the most common HLA types (*see, e.g.*, WO 94/03205; Kubo, R. T. *et al.*, *J. Immunol.* 152:3913, 1994; DiBrino, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:1508, 1993; Zhang, Q. J. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:2217, 1993). It was found that the HLA-A3 and -A11 recognized very similar motifs, leading to a hypothesis that a significant overlap might exist among their peptide binding repertoires. This was verified by a study which also demonstrated the A3 and A11 repertoires are also overlapping with those of A31, A33 and A*6801 (*see, e.g.*, Sidney, J. *et al.*, *Hu. Immunol.* 45:79-93, 1996). This specificity was defined as the A3-supertype. A significant overlap in peptide binding repertoires was also demonstrated amongst several serologically distinct HLA-B alleles (B7, B35, B51, B53 and B54) (*see, e.g.*, Sidney, J. *et al.*, *J. Immunol.* 154:247-259, 1995; Sidney, J. *et al.*, *J. Immunol.* 157:3480-3490, 1996; WO 96/03140), and amongst different A2 subtypes (*see, e.g.*, del Guercio, M-F. *et al.*, *J. Immunol.* 154:685-693, 1995; Fruci, D. *et al.*, *Hum. Immunol.* 38:187, 1993), resulting in the definition of the B7- and A2-supertypes, respectively. These supermotifs will be discussed in greater detail in the following sections.

2. The HLA-A2 Supertype

The A2 supertype will be discussed as an example of HLA supertypes and their uses. The HLA-A2 supertype includes, minimally, A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802 and A*6901. Peptide motifs have been reported for many of these alleles (*See, e.g.*, Rammensee, H. G., Friede, T., and Stevanovic, S., *Immunogenetics*, 41:178, 1995; Sudo, T. *et al.*, *J. Immunol.* 155:4749, 1995). In general, these allele-specific motifs are characterized by a preference for peptides of about 9 or 10 residues in length which bear small or aliphatic hydrophobic

residues (L, I, V, M, A, T, or Q) at position 2 and L, I, V, M, A, or T at the C terminal position, although longer and shorter peptides can bind and be recognized.

The B pocket of A2 supertype HLA molecules is characterized by a consensus motif including residues (this nomenclature uses single letter amino acid codes, where the subscript indicates peptide position) F/Y_9, A_{24}, M_{45}, E/N_{63}, K/N_{66}, V_{67}, H/Q_{70} and Y/C_{99}. Similarly, the A2-supertype F pocket is characterized by a consensus motif including residues D_{77}, T_{80}, L_{81} and Y_{116} (155). About 66% of the peptides binding A*0201 will be cross-reactive among three or more A2-supertype alleles.

The A2 supertype was defined consistent with the cross-reactivity data in the work of Fruci, *et al.* (Fruci, D. *et al.*, *Hum. Immunol.* 38:187, 1993), and from live cell binding assays (del Guercio, M.-F. *et al.*, *J. Immunol.* 154:685, 1995). More recently, Sudo and colleagues evaluated the motifs of A2-subtype alleles obtained by sequencing naturally processed peptides (Sudo, T. *et al.*, *J. Immunol.* 155:4749, 1995). Although clearly different, we noted a striking degree of similarity in the motifs, and several of the few discrete complete sequences of bound peptides obtained were actually found in multiple different A2 subtypes.

A*0207 was originally not included in the A2 supertype because of failure to detect peptide binding in live cell assays (Fruci, D. *et al.*, *Hum. Immunol.* 38:187, 1993). However, cross-reactivity between A*0207 and other A2 subtypes was detected at the functional level (Rivoltini, L. *et al.*, *J. Immunol.* 156:3882, 1996, Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997); and more recent data suggest that this HLA molecule does indeed bind a subset of the peptide repertoire bound by other A2 subtypes (Sidney, J. *et al.*, *Human Immunol.* 58:12-20, 1997).

3. The HLA-A3 Supertype

The A3 supertype will also be discussed as an example of HLA supertypes and their uses. The A3 supertype includes, minimally, A*03, A*11, A*3101, A*3301 and A*6801 alleles (Sidney, J. *et al.*, *Hu. Immunol.* 45:79-93, 1996, Sidney, J. *et al.*, *Immunol. Today* 17:261-266, 1996). A3 supertype molecules recognize a broad supermotif characterized by A, V, I, L, M, S or T in position 2, and R or K at the C-terminus. Peptide lengths of 9 to 10 amino acids have been most frequently reported, although longer and shorter peptides can bind and be recognized.

The B pocket of A3 supertype HLA molecules is characterized by the following consensus motif: M_{45}, N/K_{66}, M/V_{67}, Q/H_{66} and Y_{99}. This structural motif is similar to that for A2 supertype B pockets, which is in good agreement with the largely overlapping B pocket specificity of A2 and A3 supertype alleles. The F pocket of A3 supertype alleles is characterized by D_{77}, T_{80}, L_{81} and D_{116}. The dominant presence of a negatively charged residue (D) in positions 77 and 116 correlates with the specificity of A3-supertype alleles for peptides with a positively charged C-terminus. A*03 is the most common A3-supertype HLA molecule; about 40% of the A3-supermotif carrying peptides capable of binding A*03 display cross-reactive binding to at least three of the five most common A3-supertype HLA molecules.

4. The B7 Supertype

An additional supermotif to be discussed to exemplify the utilities of HLA supermotifs is the B7 supermotif. The B7 supertype was originally described by Sidney, *et al.* (Sidney, J. *et al.*, *J. Immunol.* 154:247, 1995; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996; Sidney, J. *et al.*, *Immunol. Today* 17:261, 1996, and in WO96/03140). The description of the supertype is consistent with results from other groups including Hill (Hill, A. V. S. *et al.*, *Nature* 360:434, 1992) and Barber (Barber, L. D. *et al.*, *Curr. Biol.* 5:179, 1995). The B7 supertype originally included B*0702, B*3501-03, B*51, B*5301 and B*5401. However, additional data for B*0703-05, B*1508, B*5501-02, B*5601-02, B*6701 and B*7801 indicate that these additional alleles should also be included within this supertype.

B7 supertype molecules share a peptide binding specificity of P in position 2 and a hydrophobic aliphatic (A, L, I, M, or V) or an aromatic (F, W, or Y) residue at the C-terminal position of the epitope which bears the motif. Modeling and X-ray crystallographic studies of the structure of B7 and B35 have been published (Huczko, E. L. *et al.*, *J. Immunol.* 151:2572, 1993; Smith, K. J. *et al.*, *Immunity* 4:203, 1996; Smith, K. J. *et al.*, *Immunity* 4:215, 1996), and offer insights into the specificity of B7 supertype HLA molecules. Structurally, the B7 supertype HLA molecules share a B pocket consensus motif of Y_9, N_{63}, I_{66}, F/Y_{67}, N/Q_{70} and Y_{99}. By contrast, no discrete B7-supertype F pocket consensus motif has yet been defined.

Peptide binding to any particular B7 supertype HLA molecule appears to occur with lower frequency, in general, than observed for A2- or A3-supertype alleles.

However, a fair degree of cross-reactivity has been noted for this supertype. About 20% of the peptides capable of binding B*0702, the most frequent B7-supertype allele, also bind 3 or more of the 5 most common B7 supertype molecules.

B. Correlation of Binding Affinity with Immunogenicity

As indicated herein, the large degree of HLA polymorphism is an important factor to be considered with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele-specific HLA molecules.

The CTL-inducing peptides that bear the supermotifs disclosed herein preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is $\leq$ 500 nM). In contrast, HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is $\leq$ 1,000 nM). Peptide binding is assessed, for example, by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in cellular screening analyses or vaccines.

High HLA binding affinity is correlated with greater immunogenicity (*see, e.g.*, Sette, *et al., J. Immunol.* 153:5586-5592, 1994; Chen *et al., J. Immunol.* 152:2874-2881, 1994; and Ressing *et al., J. Immunol.* 154:5934-5943, 1995). Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. As noted, a higher binding affinity can lead to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high or intermediate affinity binding

peptide is used. Thus, in preferred embodiments of the invention, high or intermediate affinity binding epitopes are particularly useful.

The correlation between HLA Class I binding affinity and immunogenicity was first analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al., J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold value of approximately 500 nM (more preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g.*, Schaeffer *et al., Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

In the case of tumor-associated antigens, many CTL peptide epitopes that have been shown to induce CTL that lyse peptide-pulsed target cells and tumor cell targets endogenously expressing the epitope exhibit binding affinity or IC_{50} values of 200 nM or less. In a study that evaluated the association of binding affinity and immunogenicity of a small set of such TAA epitopes, 100% (10/10) of the high binders, *i.e.*, peptide epitopes binding at an affinity of 50 nM or less, were immunogenic and 80% (8/10) of them elicited CTLs that specifically recognized tumor cells. In the 51 to 200 nM range, very similar figures were obtained. CTL inductions positive for peptide and tumor cells were noted for 86% (6/7) and 71% (5/7) of the peptides, respectively. In the 201-500 nM range, most peptides (4/5 wildtype) were positive for induction of CTL recognizing wildtype peptide, but tumor recognition was not detected.

Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205.

The current data indicate that cross-reactive peptide epitopes are frequently immunogenic epitopes, and that the converse is true, immunogenic epitopes are frequently cross-reactive. In the case of antibody molecules, high affinity correlates with low selectivity (high cross-reactivity). This is hypothesized to occur because a high affinity interaction can accommodate decreases in affinity and alterations in the

receptor/ligand interaction (Berzofsky, J. A, Berkower, I. J., and Epstein, S. L., In: *Fundamental Immunology*, 3rd ed. Paul, W. E., ed. p421, 1993). The same phenomenon is true for class II/peptide interactions, and apparently in the case of HLA class I supertypes (Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996).

As noted herein, A3- and B7-supertype specific amino acid supermotifs associated with highly cross-reactive peptides have been defined (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996). These A3 and B7 supermotifs also identify secondary anchor preferences, known to modulate binding affinity, that are shared between the HLA molecules encoded by the supertype alleles. Interestingly, these supermotifs share common features in positions 1 and 3, which in turn correlate with a known TAP transporter motif (van Endert, P. M. *et al.*, *J. Exp. Med.* 182:1883, 1995), suggesting that cross-reactive epitopes might be preferentially transported by TAP, and even that co-ordinate evolution may exist between TAP and MHC binding specificities (Sidney, J. *et al.*, *Immunol. Today* 17:261, 1996; van Endert, P. M. *et al.*, *J. Exp. Med.* 182:1883, 1995).

Taken together, these findings help explain the strong correlation between immunogenicity and HLA supertype cross-reactivity. The definition of supermotifs indicates that primary and secondary anchors can be altered (analogued) to increase cross-reactivity and immunogenicity (Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998; Parkhurst, M. R., *et al.*, *J. Immunol.* 157:2539, 1996).

C. Population Coverage

A large fraction of HLA class I, and class II molecules can be classified into a relatively few supertypes characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets. Data at the level of T cell recognition in infectious diseases and cancer further demonstrate that peptide binding properties are shared within supertypes. These studies have indicated that cross-reactive peptides are frequently recognized in the natural disease process. Such cross-reactive epitopes are recognized in the context of multiple HLA molecules, underlining the biological significance of the cross-reactivities detected at the level of binding assays.

Immunogenic peptide compositions that can provide broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the supermotif-bearing peptides of the invention (and/or nucleic acid compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table 4 shows the overall frequencies of the HLA class I supertypes in various ethnicities (Table 4a) and the combined population coverage achieved by the A2-, A3-, and B7-supertypes (Table 4b). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional peptide epitopes comprising the supermotifs of the present invention.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups.

D. Studies Validating HLA Supertypes in Infectious Diseases

Threlkeld, *et al.* (Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648-1657, 1997) reported that the same HIV RT-derived peptide was independently recognized by individuals with A3 and A11 supertype alleles chronically infected with the HIV virus. This peptide was found to bind these two alleles in a remarkably similar conformation, since CTL clones derived from the A3 patient could lyse both A3 and A11 expressing target cells (and vice versa). Threlkeld demonstrated that promiscuous T cell recognition, long recognized in the case of HLA class II molecules (Panina-Bordignon, P. *et al.*, *Eur. J. Immunol.* 19:2237, 1989), could also occur in the case of HLA class I molecules.

Khanna and co-workers (Khanna, R. *et al.*, *Eur. J. Immunol.* 28:451, 1998) have identified one EBV LMP1 protein-derived epitope recognized by CTL in the context of HLA-A2. CTL lines specific for this peptide, derived from healthy virus carriers, recognized EBV-infected B cells expressing A*0201, A*0202, A*0203, A*0204, A*0206, A*6802 and A*6901.

Bertoni, *et al.* have identified 17 different epitopes, 100% conserved in at least 80% of different HBV viral isolates, that are restricted by A2-, A3-, or B7-supertype alleles (Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503-513, 1997). Each of these epitopes was recognized by recall CTL responses of individuals that had recovered from acute self-limiting hepatitis, suggesting that all of them were generated by natural processing in the course of natural HBV infection. A population coverage approaching 90% of individuals was achieved in a real patient population with peptide epitopes collectively having three supertype specificities (supertypes A2, A3, B7). A high degree of cross-reactivity of the HBV core 18-27 epitope has also been noted in independent studies by Bertoletti, *et al.* (Bertoletti, A. *et al.*, *Hepatology* 26:1027, 1997), although a similar degree of cross-reactivity was not detected by Barouch, *et al.* (Barouch, D. *et al.*, *J. Exp. Med.* 182:1847, 1995), perhaps reflecting a difference in the sensitivity of the assay system utilized.

Finally, Doolan, *et al.* (Doolan, D. L. *et al.*, *Immunity* 7:97-112, 1997) studied the antigenicity of a total of 17 *Plasmodium falciparum* -derived A2, A3 and B7-supertype peptides in individuals immune and semi-immune to malaria. Specifically, these studies involved immune recipients of an irradiated sporozoite vaccine, and approximately two hundred individuals from malaria endemic areas. Reminiscent of the studies involving HBV epitopes, all of the cross-binding epitopes were recognized, suggesting they are indeed generated in the course of malarial infection. Furthermore, the magnitude and frequency of responses correlated with the degree of immunity, underlining the biological relevance of the epitopes identified.

E. HLA Supertypes in Cancer Immunology

A growing body of evidence also illustrates the applicability of HLA supertypes to cancer. For example in the case of the Mart-1 melanoma-associated antigen, Fleischhauer (Fleischhauer, K. *et al.*, *J. Immunol.*, 157: 787, 1996) and colleagues have reported that an immunodominant Mart-1 epitope could be presented in

the context of multiple A2 supertype alleles, including A*0201, A*0202, A*0204, A*0205, A*0206, A*0209 and A*6901.

Cellular immune responses to melanoma-associated antigens were also the focus of a study by Rivoltini and co-workers (Rivoltini, L. *et al.*, *J. Immunol.* 156:3882, 1996). A panel of Mart-1- and gp100-derived melanoma peptides bound a majority of the HLA molecules encoded by the A2 allelic subtypes, including A*0201, A*0202, A*0204, A*0205, A*0209, A*0210 and A*0211. Although not all peptide/subtype combinations were recognized, promiscuous recognition by the A*0201-restricted CTL utilized was noted for a subset of them. Kawashima, *et al.* (Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998) have shown that a majority of a set of novel CEA-, Her2/Neu- and MAGE-derived A*0201 restricted epitopes are highly cross-reactive in their binding capacity to other A2 supertype alleles.

Finally, Wang and colleagues (Wang R-F. *et al.*, *J. Immunol.* 160:890-897, 1998) reported that an A31-restricted TRP2-derived epitope was also recognized in the context of A33. Furthermore, they demonstrated that this peptide could be recognized in either context by a single TCR.

Taken together, these results validate the concept of HLA supertypes in cancer applications. Additionally, these studies suggest that dominant cancer epitopes are also, as in the case of infectious diseases, very frequently cross-reactive.

F. Definition of Additional HLA Supertypes

As discussed in the Background Section, particular progress has been made in the definition of three HLA class I supertypes, and in the identification of supermotif-bearing CTL epitopes derived from tumor-associated antigens, (*see, e.g.*, co-pending application U.S.S.N. 09/458,302) and supermotif-bearing CTL epitopes derived from pathogenic organisms, including epitopes recognized in the course of malaria, chronic HBV and HCV infections (*see, e.g.*, co-pending applications U.S.S.N. 09/239,043 and U.S.S.N. 09/350,401; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; and Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997). Disclosed herein are supermotifs that characterize cross-reactive binding by the A1, A24, B27, B58, and B62 supertypes, for use on these and other target antigens.

Two observations are made based on the HLA class I-restricted CTL epitopes identified to date. First, very few B7 supertype epitopes have been identified, probably at least in part, a reflection of the fact that B7 supermotifs are less frequent than either of the known HLA-A supermotifs (A2 and A3), and also that B7 supermotif peptides are less frequently cross-reactive than their A2- and A3-supermotif counterparts. These results reflect the need to identify additional cross-reactive epitopes. Secondly, the results illustrate that several epitope candidates binding with high affinity to HLA-A1 or -A24 have already been identified and further analysis can be performed to determine their cross-reactivity, and to identify additional high affinity binders. Thus, HLA-A2, A3, and B7 supertypes (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996; Sidney, J. *et al.*, *J. Immunol.* 154:247, 1995; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996; del Guercio, M-F. *et al.*, *J. Immunol.* 154:685, 1995; Sidney, J. *et al.*, *Human Immunol.* 58:12, 1997) have been defined. Herein we have compiled data from our own studies, and the literature, and have also performed pocket analyses on several HLA molecules and disclose, for the first time in the art, additional HLA supertypes and corresponding amino acid supermotifs.

Utilizing peptide binding motifs defined by our own and others studies, we have discovered additional HLA-A and HLA-B supertypes. We have identified these additional clusters as the A1, A24, B27, B44, B58, and B62 supertypes. The known HLA supermotifs, including those disclosed herein, are shown in Table 1. The HLA alleles comprising each of the HLA supertype families are shown in Table 5.

The following disclosure addresses the extent to which the binding repertoires of alleles within these newly identified supertypes overlap. Furthermore, analyses of HLA binding pockets allowed, in many cases, the definition of pocket sequences correlating with the super motifs. These structural motifs were then utilized to classify alleles into an appropriate supertype.

1. Motifs

Binding motifs were compiled from experimental data or from data presented in the literature and the published motif listing of Rammensee, *et al.* (Rammensee, H. G., Friede, T., and Stevanovic, S., *Immunogenetics*, 41:178, 1995), which has been updated on the internet at http://www.uni-tuebingen.de/uni/kxi/. The following methods were used in defining binding motifs: pool sequencing analyses, analysis of the binding of large libraries of peptides, single substitution analysis, and

consensus amongst known epitopes. In many cases, residues allowed within a motif are inferred on the basis of chemical similarity taking into account similar amino acid activities in previously disclosed motifs, or are predicted on the basis of pocket analysis (see below).

2. Antigens and Alleles

For the present analysis, all HLA-A and -B alleles identified through 1995 (Parham, P. *et al. Immunol. Rev.* 143:141, 1995)) were included.

In some cases, motifs have been reported for a specific allele, but not for other alleles corresponding to the same antigen and with identical peptide binding pockets. In these instances, we have drawn the conclusion that highly related alleles of the same antigen which share identical peptide binding pockets, also share identical peptide binding motifs. Upon analysis it was found that a peptide bound by an HLA molecule is also bound by a number of alleles. Typically, these alleles are structurally very closely related, and are often identical within their main peptide binding pockets. To limit redundancy in the data, only one sequence was entered for alleles, *i.e.* subtypes, with identical HLA peptide binding pockets of the same antigen (*e.g.*, serology only got to level of antigen *e.g.*, A2, but subsequent genomic analyses have identified subtypes encoded by particular alleles). The alleles sharing the same antigen and the same binding pockets have been noted in the tables.

3. Pocket Analyses

For pocket analyses, the residues comprising the B and F pockets of HLA molecules as described in crystallographic studies (Guo, H. C. *et al.*, *Nature* 360:364, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991; Madden, D. R., Garboczi, D. N. and Wiley, D. C., *Cell* 75:693, 1993), were compiled from the database of Parham, *et al.* (Parham, P., Adams, E. J., and Arnett, K. L., *Immunol. Rev.* 143:141, 1995). In these analyses, residues 9, 45, 63, 66, 67, 70, and 99 were considered to make up the B pocket, and thereby to determine the specificity for the residue in the second position of peptide ligands. Similarly, residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket, and thereby to determine the specificity for the C-terminal residue of a peptide ligand bound by the HLA molecule.

4. Population frequency of HLA supertypes

Population frequency data has been compiled from published sources (Imanishi, T. *et al.*, In: *HLA 1991, Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, Vol. 1, Ch. W15.1. K. Tsuji, M. Aizaqa, and T. Sasazuki, eds. p. 1065, 1992; Fernandez-Viña, M. A., *et al.*, *Hum. Immunol.* 33:163, 1992; Krausa, P. *et al.*, *Tissue Antigens* 45:223, 1996). As discussed above, the present analysis considers all HLA-A and B alleles for which sequence information is available. As a result, many rare alleles for which no population frequency data is available are included.

To date, most available population frequency data is at the level of the HLA antigen, but only rarely is data on allelic frequencies available. Much of this problem is alleviated, when evaluating population coverage afforded by specific HLA supertypes, by the fact that many alleles from the same antigen also share a preference for peptides that bear similar binding motifs. However, there are instances where different alleles of a given HLA molecule do not share peptide binding motifs, and it is not clear which allele is dominant. In these instances, the total population frequency of the antigen has been noted once under each of the appropriate supertypes.

Total coverage assumes Hardy-Weinberg equilibrium. The total coverage is calculated considering only those antigens or alleles experimentally confirmed to share the supertype binding preference, and therefore represents a minimal estimate. Where peptide-binding data, pool-sequencing analysis, or pocket structure based on primary sequence suggest that subtypes will have very similar, if not identical, peptide main-anchor preferences and overlapping peptide-binding specificities, a 1:1 correspondence between subtype alleles and the serologically defined antigens was assumed. As peptide binding motifs for more alleles are reported, it is conceivable that the population coverage achieved by a particular supertype could increase.

G. Novel HLA-A Supermotifs and Corresponding Supertypes

1. The HLA-A1 Supertype

Upon evaluating the pool sequencing motifs of the peptides bound by the A*0101, A*2601, and A*2602 alleles, it was discovered that they shared similar characteristics. Specifically, these alleles bound peptides having a general motif of a

small (T or S) and/or hydrophobic (L, I, V, or M) residues in position 2, and aromatic (Y, F, or W) residues at the C-terminus (DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). For two other alleles (A*2501 and A*3201) a similar motif was defined by the present inventors based on the sequences of published epitopes (van Baalen, C.A. *et al.*, *J. Gen. Virol.* 77:1659, 1996; Rickinson, A.B., Moss, D.J., *Annu. Rev. Immunol.* 15: 405, 1997; Harrer, E. *et al.*, *J. Infect. Dis.* 173:476, 1996) (Tables 6a and b).

Analysis of the B pocket polymorphic residues of these five alleles revealed that they also possessed a common pattern at the following positions: M_{45}, N_{66}, M or V_{67}, and H_{70}. In contrast, other alleles which are known to present a different specificity for position 2, such as B7, B27, B44, and A24, matched this consensus motif only partially, or not at all (Table 6a), thus these are not included in the A1 supertype.

It is also evident the residues comprising the B pocket of the A1-supertype alleles are very similar to those of the alleles in the A2- and A3-supertypes. This is not surprising in that alleles of all three groups prefer aliphatic or hydrophobic residues in position 2 of their peptide ligands. Thus, A1-supertype alleles all possess residues which fit within a larger HLA-A B pocket motif describing alleles binding small and/or hydrophobic residues at position 2.

Inspection of the F pocket of A1 supertype alleles defined two separate motifs: N_{77}-T_{80}-L_{81}-D or N_{116} (A*0101, A*2601, and A*2602), and S_{77}-I_{80}-A_{81}-D_{116} (A*2501 and A*3201) (Table 6b). These two motifs are not found in any other molecule encoded by an HLA allele with a known F pocket specificity which is different than the A1-like specificity for aromatic residues.

Examination of the sequences of HLA-A and B alleles for which no peptide binding motif is known revealed that A*0102, A*2604, A*3601, A*4301, and A*8001 possess A1-supertype B pocket consensus motifs (Table 6a). These alleles also exactly match A*01 and A*2601 in the F pocket (Table 6b,c). On the basis of these shared structural features, A*0102, A*2604, A*3601, A*4301, and A*8001 are included as members of the HLA-A1 supertype family. Exemplary members of the A1 supertype family, both verified and those that are included based on pocket structure, are set out in Table 5.

Listed in Table 6c are the phenotypic frequencies of A1-supertype alleles in five major ethnic populations. Coverage has been calculated as described above. As

shown, the A1 supertype is represented with an average frequency of 25.2%, ranging from a low of 14.7% in Hispanics, to a maximum of 47.1% in Caucasians.

Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

2. The HLA-A24 Supertype

Upon evaluating the published pool sequencing motifs for A*2402 and A*3001 (Kubo, R. T. *et al., J. Immunol.* 152:3913, 1994; Maier, R. *et al., Immunogenetics* 40:306, 1994) and analyzing published epitopes recognized by A*2301 (Koziel, M. J. *et al., J. Clin. Invest.* 96:2311, 1995; Khanna, R. *et al., J. Virol.* 70:5357, 1996), the present inventors determined that these alleles share an overlapping specificity for aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue in position 2 and Y, F, W, L, I, or M at the C-terminus (Tables 7a and b).

A*2301 and A*2402 share an identical B pocket structural motif: S_9-M_{45}-E_{63}-K_{66}-V_{67}-H_{70}. The B pocket structure of A*3001 differs from that of A*2301 and A*2402 at 3 positions: F_9, N_{66}, and Q_{70}. Neither of these structural motifs are found in alleles with different B pocket specificities (Table 7a).

The presence of a small residue (S) in position 9, as opposed to the F or Y present in most other HLA-A alleles, is hypothesized to allow A*2301 and A*2402 to accommodate large aromatic residues in position 2 of their peptide ligands. It is more difficult to speculate on the structural features imparting the A*3001 specificity. However, it is noted that the A*3001 B pocket matches that of A*0301, which has also been reported to accommodate a broad range of residues in position 2 of its peptide ligands (Kubo, R. T. *et al., J. Immunol.* 152:3913, 1994; Sidney, J. *et al., Hu. Immunol.* 45:79, 1996).

Analysis of the F pocket of these residues revealed that the structures of A*2301 and A*2402 are identical: N_{77}-I_{80}-A_{81}-$Y_{11}6$ (Table 7a). This motif is also found in HLA-B alleles (B*5101-05, B*5201, and B*5702), which share the same C-terminal (although not the same B pocket) specificity (Sidney, J. *et al., J. Immunol.* 157:3480, 1996; Barber, L. D. *et al., J. Immunol.* 158:1660, 1997; Falk, K. *et al. Int. Immunol.* 7:223, 1995). The F pocket structure of A*3001 (D_{77}-T_{80}-L_{81}-H_{116}) is again somewhat

different from that of A*2301 and A*2402, and is indeed unique among HLA alleles: no other alleles sequenced to date possess H in position 116.

Of alleles for which no peptide binding motif is known, the A*24 and A*30 subtypes A*2403, A*2404, A*3002, and A*3003 possess B and F pocket structures identical (or conservatively similar) to the A24 supertype alleles. These alleles have been included within the A24-supertype. Exemplary members of the A24 supertype family, both verified and those that are included based on pocket structure, are set out in Table 5.

The A24-supertype is represented in 5 major ethnic populations with an average frequency of 40.0%, ranging from a low of 23.9% in Caucasians, to a maximum of 58.6% in Japanese (Table 7c).

Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif..

H. Novel HLA-B Supermotifs and Corresponding Supertypes

The HLA-B loci is apparently undergoing rapid evolution, and is much more polymorphic than the A loci (Parham, P., Adams, E. J., and Arnett, K. L., *Immunol. Rev.* 143:141, 1995; Watkins, D. I. *et al.*, *Nature* 357:329, 1992; McAdam, S. N. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:5893, 1994). However, when all of the known HLA-B motifs were compiled, it was noted that all B alleles, regardless of the structure of their F pocket, bound peptides with hydrophobic C-termini. Furthermore, the hydrophobic specificity permitted, in most cases, both aliphatic and aromatic residues. In contrast to the situation with the HLA-A alleles analyzed above, classification of HLA-B alleles on the basis of F pocket motifs has not been possible. Instead, it appeared that the functional effect (in terms of peptide binding) of HLA-B polymorphism is on the B pocket. We determined HLA-B B pocket consensus motifs, corresponding to the specificity for the residue in position 2 of peptide ligands, for each of the supertypes described below. For these reasons, in the ensuing discussion of HLA-B supertypes, the discussion of structural motifs will be largely focused on the B pocket.

1. The HLA-B27 Supertype

Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least B*1401, B*1402,

B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 supertype are shown in Table 5.

Pool sequencing motifs for B*1401, B*1402, B*1503, B*1509, B*1510, B*1518, B*3801, B*3802, B*3901, B*3902, B*3903, B*3904, B*4801, B*4802, B*7301, B*2701, B*2702, B* 2703, B*2704, B*2705, B*2706, B*2707, and B*2708 were analyzed and discovered to share a specificity for peptides with positively charged (R, H, K) residues in position 2, and hydrophobic (A, L, I, V, M, Y, F, W) residues at the C-terminus (Tables 8a and b) (Barber, L. D. *et al.*, *J. Immunol.* 158:1660, 1997; DiBrino, M. *et al.*, *J. Biol. Chem.* 269:32426, 1994; Rötzschke, O. *et al.*, *Immunogenics* 39:74, 1994; Garcia, F. *et al.*, *Tissue Antigens* 49:215, 1997; Jardetzky, T. S. *et al.*, *Nature* 353:326, 1991; Falk, K. *et al.*, *Immunogenetics* 41:162, 1994; Barber, L. D., Percival, L., and Parham, P., *Tissue Antigens* 47:472, 1996; Boisgérault, F. *et al.*, *J. Clin. Invest.* 98:2764, 1996).

Analysis of the relevant B pocket residues of these alleles (Table 8a) reveals a consensus motif of E in position 45, and either C or S in position 67. E in position 45 is also seen in some alleles of the B7-supertype, but only in combination with F or Y in position 67. Similarly, S in position 67 is seen also in B44- and B62-supertype alleles (see below), but never in combination with E in position 45. C in position 67 is exclusive to B27-supertype alleles.

Functionally, it is speculated that the E in position 45 dominates in determining the specificity for positively charged residues in position 2 of peptide ligands. The effect of E_{45} is apparently augmented by the presence of the small residue, C or S, in position 67. All B7 supertype alleles possessing E in position 45 also have, as noted above, a bulky aromatic residue in position 67.

The structure of the F pockets for B*1503, B*1510, B*1518, B*4801, and B*4802are also consistent with those of other HLA-B alleles binding peptides with hydrophobic C-termini (Table 8b). These alleles have been included within the B27-supertype. Exemplary members of the B27 supertype family, both verified and those that are included based on pocket structure, are set out in Table 5.

As shown in Table 8c, the B27-supertype is represented with an average frequency of 23.4% in five major ethnic populations. The frequency ranges from a low of 13.3% in Japanese, to a maximum of 35.3% in Hispanics.

Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

2. The HLA-B44 Supertype

A shared specificity for peptides with negatively charged (D, E) residues in position 2, and hydrophobic residues at the C-terminus, was discovered on the basis of published pool sequencing motifs for B*3701, B*4402, B*4403, B60 (B*4001), and B61 (B*4006) (Tables 9a and b) (Falk, K. *et al.*, *Immunogenics* 38:161, 1993; Fleischhauer, K. *et al.*, *Tissue Antigens* 44:311, 1994; Thorpe, C. J., and Travers, P. J., *Immunogenetics* 40:303, 1994; DiBrino, M. *et al.*, *Biochemistry* 34:10130, 1995; Harris, P. E. *et al.*, *J. Immunol.* 151:5966, 1993; Falk, K. *et al.*, *Immunogenetics* 41:165, 1995). One B18 restricted epitope (EBV 397-405, sequence DEVEFLGHY) has been reported in the literature (Steven, N. M. *et al.*, *J. Exp. Med.* 185:1605, 1997) which suggests that the HLA molecules encoded by B*18 alleles also share this specificity.

Table 9a lists the relevant B pocket residues of these alleles. With the exceptions of B*18 and B*3701, all B44 supertype alleles possess K in position 45. The occurrence of a positively charged residue here is unique amongst HLA alleles, and constitutes the most immediate B44-supertype B pocket consensus motif. As with the B27 supertype, we hypothesize it is the residue in position 45 which dictates the B pocket specificity of these alleles, and that this charge is probably made available to peptide ligands by the simultaneous presence of S in position 67.

The ability of B*3701 to bind negatively charged residues in the B pocket, even though it does not possess a positive charge in position 45, is perhaps explained by the unique presence of S in position 99. This small polar residue, in conjunction with the positively charged residue (H) in position 9 and small polar residue (T) in position 45, may make the pocket environment sufficiently polar to bind negatively charged residues. Similarly, the B pocket specificity of B*18 alleles, which also do not have K in position 45, may be rationalized from the nature of its variations. These alleles possess N in position 63, as opposed to the E present in other B44-supertype alleles, in conjunction with the positively charged H in position 9 and small polar T in position 45.

HLA-B alleles for which no peptide binding motif is known but which possess a K in position 45 include B*4101, B*4501, B*4901, and B*5001. In fact, the B

pocket structure of these alleles is identical to B*4001. The structure of their F pockets also correspond with those of other HLA-B alleles binding hydrophobic C-termini. On the basis of these observations, they have been tentatively included within the B44-supertype. The B44-supertype is present across the five major ethnic populations with an average frequency of 37.0%, with a low of 21.2% in Blacks, and a maximum of 43.0% in Hispanics (Table 9c).

Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

3. The HLA-B58 Supertype

Upon evaluating pool sequencing motifs for B*1516, B*1517, B*5701, B*5702, B*5801, and B*5802, it was determined that there is a shared preference for small aliphatic residues (A, S, or T) in position 2, and aromatic or hydrophobic (F, W, Y, L, I, V, M, or A) residues at the C-terminus of their peptide ligands (Tables 10a and b) (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999 for reviews of relevant data).

The B pocket structures of these alleles (Table 10a), indicate a consensus motif of Y_9-N_{66}-M_{67}-S_{70}. This motif is entirely unique to B58-supertype alleles. In terms of function, it is hypothesized that the M present in positions 45 and 67 precludes the binding of bulkier side chains. Only A*0101 and A*3601 have the M_{45}-M_{67} motif, and these alleles are also associated with a preference for small residues in position 2. The presence of Y_9, as opposed to F, may explain the inability of B58-supertype molecules to accommodate the somewhat larger range of residues allowed in position 2 by A*0101. Exemplary members of the B58 supertype family are set out in Table 5.

The B58-supertype is represented in the Black population with a frequency of 25.1%. Overall, it is represented, on average, in the five major ethnic populations with a frequency of 10.3% (Table 10c).

Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

4. The HLA-B62 Supertype

Pool sequencing motifs have been published for B46, B*5201, B*1501 (B62), B*1502 (B75), and B*1513 (B77). Evaluation of these data led to the discovery that there was a shared preference for ligands with the polar aliphatic residue Q, or a hydrophobic aliphatic residue (L, V, M, I, or P), in position 2; and a hydrophobic residue (F, W, Y, M, I, V, L, or A) at the C-terminus (Barber, L. D. *et al.*, *J. Immunol.* 158:1660, 1997; Falk, K. *et al. Int. Immunol.* 7:223, 1995; Falk, K. *et al.*, *Immunogenetics* 41:165, 1995; Prilliman, K. *et al.*, *Immunogenetics* 45:379, 1997; Barber, L.D. *et al.*, *J. Exp. Med.* 184:735, 1996) (Tables 11a and b).

Upon analysis of the relevant B pocket residues for these alleles, a consensus motif of Y_9-M/T_{45}-I_{66}-S_{67}-N_{70} (Table 11a) was identified. This motif is similar to that found in some B44- and B27-supertype alleles, except B62-supertype alleles do not possess a charged residue at position 45.

B*1301, B*1302, B*1506, B*1512, B*1514, B*1519, and B*1521 are additional HLA-B alleles which match the B62-supertype B pocket consensus motif, and which have F pocket structures consistent with a hydrophobic specificity (Tables 11a and b). Thus, these alleles have been included within this supertype. Exemplary members of the B62 supertype family, both verified and those that are included based on pocket structure, are set out in Table 5.

Listed in Table 11c are the phenotypic frequencies of B62-supertype alleles in the five major ethnic populations. As shown, the B62-supertype is represented with an average frequency of 18.1%, ranging from a low of 4.8% in Blacks, to a maximum of 36.5% in Japanese.

It should be noted that this motif type has similarities to those of the A2- and A24-supertypes. Thus, additional coverage may be achieved by including some or all of these alleles in the A2- and/or A24-supertypes.

Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

I. Population Coverage

Together, the present analysis indicates that six additional HLA-supertypes exist. Table 4 summarizes these supertypes, and indicates an estimate of their prevalence

in five major ethnic groups. The B44-, A1-, and A24-supertypes are present, on average, in a range from 25% to 40% in the five major ethnic populations. While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one of the five major ethnic groups. Table 4b shows the population coverage achieved by the A2-, A3-, and B7-supertypes, and the additional coverage obtained by the addition of A1-, A24, and B44-supertypes, or all of the supertypes described herein. As shown, by including epitopes from the six most frequent supertypes, an average population coverage of 99.3% is obtained for five major ethnic groups.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. Focusing only on six supertypes affords population coverage in the 98.1 to 100% range for all major ethnic populations.

J. Evaluation of Immunogenicity

Upon identification of peptide sequences comprising a supermotif of the invention, the corresponding peptide can be obtained or synthesized and tested for immunogenicity and cross-reactive binding capabilities. A variety of assays to detect and quantify the affinity of interaction between peptide and MHC have been established (Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J., *Curr. Biol.* 6:52, 1994; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994); and a threshold of affinity associated with generation of an immune response has also been elucidated (*see, e.g.*, Section C. of the present application; also Schaeffer, E. B. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:4649, 1989). Thus, by a combination of motif searches and MHC-peptide binding assays, potential candidates for epitope-based vaccines can be identified.

Various strategies can be utilized to evaluate immunogenicity and to identify immunogenic epitopes, including:

1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.*, Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of

antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a ^{51}Cr-release assay involving peptide-sensitized target cells.

2) Immunization of HLA transgenic mice (*see, e.g.*, Wentworth, P. A. *et al.*, J. Immunol. 26:97, 1996; Wentworth, P. A. *et al.*, Int. Immunol. 8:651, 1996; Alexander, J. *et al.*, J. Immunol. 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured in vitro in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, e.g., a ^{51}Cr-release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

3) Demonstration of recall T cell responses from patients who have been effectively vaccinated or who have a tumor; (*see, e.g.*, Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997; Tsang *et al.*, *J. Natl. Cancer Inst.* 87:982-990, 1995; Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996). In applying this strategy, recall responses are detected by culturing PBL from patients with cancer who have generated an immune response "naturally", or from patients who were vaccinated with tumor antigen vaccines. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce in vitro primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon-γ release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp. Med.* 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

K. Immunogenic Epitopes as Vaccines

Vaccines that contain an immunogenically effective amount of one or more supermotif-bearing peptides as described herein are a further embodiment of the invention. Once appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (*e.g.*,Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (*see, e.g.*, Eldridge, *et al.*, *Molec. Immunol.* 28:287-294, 1991: Alonso *et al.*, *Vaccine* 12:299-306, 1994; Jones *et al.*, *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (*see, e.g.*, Takahashi *et al.*, *Nature* 344:873-875, 1990; Hu *et al.*, *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (*see e.g.*, Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S.

H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al.*, *J. Immunol. Methods.* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the supermotif-bearing peptide(s). The peptide(s) can be individually linked to its own carrier; alternatively, the peptide(s) can exist as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune response. The composition may be a naturally occurring region of an antigen or may be prepared, *e.g.*, recombinantly or by chemical synthesis.

Useful carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein,

CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS).

Upon immunization with a composition that includes a supermotif-bearing peptide in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some instances it may be desirable to combine the class I peptide vaccines of the invention with vaccines which induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to surface antigens; and/or to HLA class II epitopes. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142).

A peptide bearing a supermotif as described herein may be incorporated in a given vaccine composition with one or more additional epitoes. The multiple epitopes, may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived. Furthermore, the multiple epitopes may constitute combinations of CTL and HTL epitopes that can be derived from the same, or different, antigens. A peptide that comprises a supermotif-bearing epitope of the invention and one or more additional peptides may be of a variety of lengths, but preferably does not comprise an entire native antigen. Such peptides may be 250, and are often 100, 50, 25, 15, or 11, 10, 9, or 8 amino acids in length.

Furthermore, any of these embodiments can be administered as a nucleic acid mediated modality. The epitopes identified that bear the supermotifs of the invention can be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, for example, as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected host

or into a non-infected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, *e.g.* adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see*, *e.g.*, U.S. Patent No. 5,922,687).

The antigenic peptides idenitifed as described herein are used to elicit a CTL response *ex vivo*, as well. *Ex vivo* administration is described, for example, in WO 98/33888. The resulting CTL cells, can be used to treat chronic infections, or tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL responses to a particular antigen (infectious or tumor-associated antigen) are induced by incubating in tissue culture the patient's, or genetically compatible, CTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 14 weeks), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells. Alternatively, dendritic cells are transfected, *e.g.*, with a minigene construct in accordance with the invention, in order to elicit immune responses.

Vaccine compositions may also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

EXAMPLES

Example 1: Establishment of Appropriate HLA Class I Peptide Binding Assays and the Evaluation of Binding of Peptides to Multiple Alleles within a Supertype

In order to validate the HLA class I supertypes disclosed for the first time herein, a complete panel of HLA binding assays specific for the various alleles within the supertypes targeted (the A1-, A24-, B27-, B58-, and B62- supertypes) is useful, (*see* Tables 6, 7 and 9 for summary). The rationale for focussing on these supertypes is that they represent, along with the already well characterized A2-, A3- and B7-supertypes, the supertypes. Definition of these specificities allows one to provide truly multispecific responses, and population coverage of 95% or more.

1. A1-Like Alleles

As detailed above, alleles of the A1-supertype (which minimally comprises the A*0101, A*2501, A*2601, A*2602, and A*3201 alleles) share a B pocket specificity for aliphatic hydrophobic residues (T, I, L, V, M, or S) in position 2 of peptide ligands, and an F pocket specificity for aromatic residues (F, W, or Y) at the peptide C-terminus. First, we examined whether these alleles also share overlapping peptide binding repertoires. For this purpose it is important to define the binding specificity of these alleles, and the nature of their repertoire.

For A*0101, an optimized peptide binding assay which uses purified MHC, has already been established, and its peptide binding specificity has been characterized in detail (Kubo, R. T. *et al.*, *J. Immunol.* 152:3913, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Accordingly, reliable binding assays were established for A*2501, A*2601-02 and A*3201. To this end, the strategy used is the one previously applied to establish over forty different binding assays specific for human or mouse MHC, class I or class II (Sidney, J. *et al.*, In: *Current Protocols in Immunology*, unit 18.3, 1998; Sette, A. *et al.*, In: *Immunology Methods Manual.* Ed.T. Picknett, p. 649, 1997). Briefly, in these assays, purified MHC and a radiolabeled high affinity ligand are

incubated for two days at room temperature, in presence of a cocktail of protease inhibitors. After incubation, the MHC-peptide complexes are separated from unbound peptide by gel filtration, and the amount of bound/free radioactivity is determined. When the assay is run in presence of different amounts of an unlabeled competitor peptide, the 50% inhibitory ($IC_{50\%}$) dose is determined. Under appropriate assay conditions, the $IC_{50\%}$ provides an effective approximation of K_D.

EBV-transformed homozygous cell lines are utilized as sources of purified HLA molecules. Table 12 lists a number of lines expressing A1-, A24-, or B44-supertype molecules known to be available to the scientific community. Remaining cell lines are obtained by contacting appropriate laboratories and repositories (*e.g.*, ASHI; see also ref. Bodmer, J. G. *et al.*, *Human Immunol.* 53:98, 1997).

To establish assays for supertype alleles, high affinity ligands are identified. As a source of high affinity ligands, published data regarding naturally processed peptides or epitopes is utilized to identify suitable ligands, or to generate idealized "poly-A" or "consensus" peptides that are capable of binding A*2501, A*2601-02 and A*3201. The peptide can subsequently be used to define a motif; if the new motif is compatible with the A1 motif, highly cross-reactive peptides are identified by screening HLA-A1 binding peptides for their binding capacity for these other A1-supertype alleles.

The binding repertoire of additional alleles predicted to share A1-supertype specificity, such as A*3601, A*4301 and A*8001 is also analyzed by utilizing EBV-transformed HLA class I transfected cell lines as a source of HLA, and highly cross-reactive peptides identified in the previous series of experiments as radiolabelled ligands.

2. A24 Supertype

The peptide binding specificity of the A24-supertype comprises F, W, Y, L, I, V, M, or T in position 2; and Y, F, W, L, I, or M at the C-terminus. As described herein (see, *e.g.*, Tables 7a-c), the A24-supertype minimally includes the A*2301, A*2402, and A*3001 HLA alleles. A reliable peptide binding assay specific for A*2402 was established in our laboratory using the EBV-transformed KT3 homozygous cell line and a pool sequencing consensus motif analog peptide (sequence AYIDNYNKF) as the radiolabeled ligand. A suitable signal, *e.g.*, fifteen percent binding in this system, may be obtained using approximately 20 nM purified MHC; the IC_{50} of the radiolabeled peptide

is 12 nM. A detailed analysis of the peptide binding specificity of A*2402 has been described (Kondo, A. *et al.*, *J. Immunol.* 155: 4307, 1995).

Purified A*2301 and A*3001 molecules are obtained from appropriate EBV-transformed homozygous cell lines; candidate lines are listed in Table 12. Using methodology analogous to that used to establish HLA-A1-supertype binding assays, naturally processed peptides, known epitopes, poly-A analogs, or pool sequencing consensus motif peptides are assessed; and candidates for use as high affinity radiolabeled ligands are identified. Peptides binding A*2402, the most frequent A24-supertype allele, are assessed for their capacity to additionally bind A*2301 and A*3001 to identify cross-reactive peptides.

3. B44 Alleles

The B44-supertype comprises B*1801-02, B*3701, B*4402-04, B*4001 (*i.e.*, B60), B*4002 and B*4006 (*i.e.*, B61). As detailed above, these alleles recognize peptides with a negatively charged residue (E, or D) in position 2, and a hydrophobic residue at their C-termini. Initially, one assay representing each antigen (B18, B37, B44, B60, and B61) is established, as outlined above, using candidate cell lines listed in Table 12. For example, an assay for B*4403, the most frequent B44-supertype antigen, was established using an analog of a naturally processed peptide (EF-1; sequence AEMGKYSFY) identified by Fleischhauer, *et al.* (Fleischhauer, K. *et al.*, *Tissue Antigens* 44:311, 1994) as the radiolabeled ligand, and the PITOUT cell line as the source of MHC. In this system, a suitable signal, *e.g.*, 15% binding of the radiolabeled peptide, is achieved using approximately 100 nM of purified MHC; the IC_{50} of the radiolabeled ligand is 62 nM.

Assays for B18, B37, B60, and B61 are established and characterized consistent with corresponding protocols herein for other supertypes; peptides recognized by B*4403 are analyzed, as appropriate, for their capacity to bind those alleles determined to recognize the B44-supertype specificity. In a second phase, assays for alleles predicted on the basis of pocket analysis to have a B44-supertype specificity are established and characterized.

Peptide binding to each of the B44 allele-specific HLA molecules can be modulated by substitutions at anchor positions.

4. B27 Alleles

The HLA-B27 supertype minimally comprises the B*1401, B*1402, B*1503, B*1509, B*1510, B*1518, B*3801, B*3802, B*3901, B*3902, B*3903, B*3904, B*4801, B*4802, B*7301, and B*2701-08 HLA alleles. As detailed above, the B27 supertype HLA molecules recognize peptides characterized by the presence of positively charged (R, H, or K) residues as primary anchors at position 2 and hydrophobic (A, L, I, V, M, Y, F, or W) residues as primary anchors at the C-terminal position.

Initially, one assay representing each antigen (B*1401, B*1402, B*1503, B*1509, B*1510, B*1518, B*3801-02, B*3901-04, B*4801-02, B*7301, and B*2701-08) is established, as outlined above, using appropriate cell lines. For example, an assay for B*2705 was established using a peptide (FRYNGLIHR) as the radiolabeled ligand, and the LG2 cell line as the source of MHC. In this system, 15% binding of the radiolabeled peptide is achieved using approximately 150 nM of purified MHC; the IC_{50} of the radiolabeled ligand is 64 nM.

Assays for the other member of the supertype family are also established and characterized in accordance with these parameters. Purified HLA molecules are obtained from appropriate EBV-transformed homozygous cell lines. Using methodology analogous to that used to establish HLA-A1-supertype binding assays, naturally processed peptides, known epitopes, poly-A analogs, or pool sequencing consensus motif peptides are assessed, and candidates for use as high affinity radiolabeled ligands are identified. Using the allele-specific assays, peptides binding the most frequent B27-supertype allele are assessed for their capacity to bind to additional B27 supertype family members to identify cross-reactive peptides.

Peptide binding to each of the B27 allele-specific HLA molecules can be modulated by substitutions at anchor positions.

5. B58 Alleles

The HLA-B58 supertypes comprises B*1516, B*1517, B*5701, B*5702 and B*5801 and B*5802. As detailed above, the B58 supermotif is characterized by the presence in peptide ligands of small aliphatic residues (A, S, or T) as primary anchor residues at position 2 and aromatic or hydrophobic residues (F, W, Y, L, I, V, M, or A) as primary anchor residues at the C-terminal position. Initially, one assay representing each antigen (B*1516, B*1517, B*5701, B*5702, and B*5801-02) is established, as outlined

above, using appropriate candidate cell lines expressing the respective antigen. For example, an assay for B*5701 was established using a B*58 supermotif-bearing peptide as the radiolabeled ligand, and an available cell line as the source of MHC B*5701. In this assay system, 15% binding of the radiolabeled peptide is achieved to obtain a suitable signal by titrating reactant amounts in accordance with procedures in the art, and the IC_{50} of the radiolabeled ligand is determined.

Assays for the other member of the supertype family are also established and characterized. Purified HLA molecules are obtained from appropriate EBV-transformed homozygous cell lines. Using methodology analogous to that used to establish HLA-A1-supertype binding assays, naturally processed peptides, known epitopes, poly-A analogs, or pool sequencing consensus motif peptides are assessed, and candidates for use as high affinity radiolabeled ligands are identified. Using allele-specific assays, peptides binding the most frequent B58-supertype allele are assessed for their capacity to bind to additional B58 supertype family members to identify cross-reactive peptides.

Peptide binding to each of the B58 allele-specific HLA molecules can be modulated by substitutions at anchor positions.

6. B62 Alleles

The HLA-B62 supertype minimally comprises the B*1501, B*1502, B*1513, and B*5201 HLA alleles. As detailed above, the B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or L, I, V, M, or P as a primary anchor in position 2 and hydrophobic residue (F, W, Y, M, I, or V) as a primary anchor at the C-terminal position. Initially, one assay representing each antigen (B*1501, B*1502, B*1513, and B*5201) is established, as outlined above, using candidate cell lines. For example, an assay for B*1501 is established using a peptide that bears the B62 supermotif as the radiolabeled ligand, and an available cell line that expesses B*1501 as the source of MHC. In this system, 15% binding of the radiolabeled peptide is achieved using titration procedures known in the art, and the IC_{50} of the radiolabeled ligand is determined.

Assays for the other member of the supertype family are also established and characterized.. Purified HLA molecules are obtained from appropriate EBV-transformed homozygous cell lines. Using methodology analogous to that used to

establish HLA-A1-supertype binding assays, naturally processed peptides, known epitopes, poly-A analogs, or pool sequencing consensus motif peptides are assessed, and candidates for use as high affinity radiolabeled ligands are identified. Using allele-specific assays, peptides binding the most frequent B62-supertype allele are assessed for their capacity to bind to additional B62 supertype family members to identify cross-reactive peptides.

Peptide binding to each of the B62 allele-specific HLA molecules can be modulated by substitutions at anchor positions.

Example 2: Screening of Protein Sequences from Disease Targets for Supermotif Containing Peptides

In this example, the frequency that cross-reactive binding is observed for HLA molecules encoded by alleles of a given supertype is determined. It is also determined whether any particular combinations of anchor residues in position 2 and at the C-terminus, or whether a particular size (*e.g.*, 8-mer, 9-mer, 10-mer, or 11-mer) is preferentially associated with cross-reactive binding. The approach involves screening available protein sequences for the occurrence of motif-containing peptides. These peptides are then analyzed for their capacity to bind to multiple allele-specific HLA molecules from a particular supertype.

Computer programs that allow the rapid screening of protein sequences for the occurrence of the 6 supermotifs described herein have already been and/or readily are established. These programs are employed to identify epitopes in a known or newly discovered sequence of amino acids, or encoded by a known or newly discovered nucleic acid sequence. Such programs are also used to generate epitope analogs and/or nucleic acids which encode epitope analogs. Also see, Example 3 herein.

For example, the target molecules considered have included all of the HBV proteins (surface, core, polymerase, and X), the entire HCV polyprotein, and four proteins from *P. falciparum* circumsporozoite and liver stage forms (CS, SSP2, LSA1, and EXP1). These proteins yield a database of sufficient size (e.g. >200 motif positive peptides) and therefore allow definition of those features associated with cross-reactive binding to MHC alleles within a supertype (Gulukota, K. *et al.*, *J.Mol.Biol.* 267:1258, 1997).

In addition to identifying particular peptides that demonstrate cross-reactivity, these cross-reactive peptides are analyzed and the structural characteristics correlated with supertype cross-reactivity are determined. The strategy is essentially as employed previously for defining the rules of A2-, A3- and B7-supertype cross-reactivity (see, *e.g.*, Sidney, J. *et al.*, *Human Immunol.* 45:79, 1996; Sidney, J. *et al. J. Immunol.* 154:247, 1995; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996; delGuercio, M.-F. *et al.*, *J. Immunol.* 154:685, 1995; Sidney, J. *et al.*, *Human Immunol.* 58:12, 1997)). Peptides are analyzed and screened for the absence of residues (either primary anchor or secondary anchors) that are associated with weak binding to the HLA molecules encoded by individual supertype member alleles in question and/or associated with poor cross-reactivity. An analogous approach allows the identification and screening for the presence of residues associated with good binding and/or good cross-reactivity for multiple HLA molecules encoded by various alleles from a particular supertype.

For example, particular combinations of primary residues such as L_2V_c for the A2 supertype, V_2K_c for the A3-supertype and P_2I_c for the B7 supertype have been associated with high cross-reactivity (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996; Sidney, J. *et al.*, *Human Immunol.* 58:12, 1997) (in this nomenclature the subscript denotes the amino acid position in a peptide epitope). Favorable combinations of secondary anchors have also been identified following this approach (for example, Y, F and W at position 1 were associated with high affinity binding for both A3 and B7 supertype molecules (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996)).

<u>Example 3:</u> Analog Preparation: Alteration of Peptide Structure at Primary or Secondary Anchor Residues

Although peptides with suitable cross-reactivity among alleles of a supertype are identified by the screening procedures described herein, cross-reactivity is not always complete and in such cases manipulations to further increase cross-reactivity can be useful. It can also be useful to manipulate the sequence to alter, generally increase, binding affinity to one or more HLA molecules as described, for example, in co-pending U.S.S.N. 09/226,775. It is to be noted that modifications can be done to achieve other objectives such as descreased binding affinity or lessened cross-reactivity.

The strategy employed herein entails defining the rules of cross-reactive binding, preferably using the methods utilized to find peptides recognized by the A2-, A3- and B7-supertypes (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996; Sidney, J. *et al.*, *J. Immunol.* 154:247, 1995; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996; del Guercio, M-F. *et al.*, *J. Immunol.* 154:685, 1995; Sidney, J. *et al.*, *Human Immunol.* 58:12, 1997). Accordingly, particular combinations of primary anchor residues which allow for maximum cross-reactivity are considered. Having established the general rules that govern cross-reactivity and/or affinity of peptides for MHC alleles of a given supertype, modification (*i.e.*, analoguing) of the structure of peptides of particular interest in order to achieve broader HLA binding capacity are performed. Typically, peptides which exhibit the broadest crossreactivity patterns, are selected for further analysis.

This type of "anchor fixing" has proven highly successful in numerous studies (*see, e.g.*, Parkhurst, M. R., *et al.*, *J. Immunol.* 157:2539, 1996; Parkhurst, M. R., *et al.*, *J. Immunol.* 157:2539, 1996; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996), and has a minimized probability of perturbing T cell recognition of peptide/HLA complexes. For example, when a preferred amino acid is not already present, it is added by substitution or if a deleterious residue is present it is removed by substitution.

For example, B7-supertype epitopes were modified by substitution with I at the C-terminal residue (Sidney, J. *et al.*, *J. Immunol.* 157:3480, 1996); A3-supertype epitopes were modified to bear V in position 2, and either R or K at the C-terminus (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996), where such residues were not already present.

Next, removal of residues that are detrimental to binding or cross-reactivity ("deleterious" residues) is considered. For example, in the case of the A3 supertype, when all peptides that have such negative residues are removed from the analysis, the incidence of cross-reactivity increases from 22% to 37% (Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete the negative residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). Overall, an enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, residues associated with high affinity binding to multiple alleles within a superfamily are inserted.

There is a possibility that changes in the epitope may impact the ability of T cells to recognize the original native epitope when presented in a peptide/HLA complex. In vaccine design, it is generally preferred to only induce an immune response that will target one or more epitopes presented in an actual disease state; *i.e.,* it is preferred to induce an immune response that targets a "wild type" peptide even if this response is induced by an analog. For similar reasons, it is generally not preferred to induce an immune response to a cryptic wild type epitope, since these generally are not available to the immune system *in vivo*. To evaluate the possibility that the immune response induced no longer targets the wild type epitope, the analog peptide is used to immunize cells *in vitro* from individuals of the appropriate HLA allele, and the ability to induce lysis of wild type peptide sensitized target cells is evaluated. Also used as targets are cells that have been either infected or transfected with the appropriate genes to establish whether endogenously produced antigen is also lysed by these CTL. Only those variants that induce immune responses capable of lysing APC presenting wild type peptide will be considered as potential candidates for the improved specificity for the antigen and/or cross-reactivity among HLA molecules encoded by members of a supertype.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

TABLES

Table 1

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	**T***LVMS*		**FWY**
A2	**LIVM***ATQ*		**IV***MATL*
A3	**VSMA***TLI*		**RK**
A24	**YF***WIVLMT*		**FI***YWLM*
B7	**P**		**VILF***MWYA*
B27	**RHK**		**FYL***WMIVA*
B44	**E***D*		**FWYLIMVA**
B58	**ATS**		**FWY***LIVMA*
B62	**QL***IVMP*		**FWY***MIVLA*
MOTIFS			
A1	**TSM**		**Y**
A1		**DE***AS*	**Y**
A2.1	**LM***VQIAT*		**V***LIMAT*
A3	**LMVISATF***CGD*		**KYR***HFA*
A11	**VTMLISAGN***CDF*		**K***RYH*
A24	**YFWM**		**FLIW**
A*3101	**MVT***ALIS*		**R***K*
A*3301	**MVALF***IST*		**RK**
A*6801	**AVT***MSLI*		**RK**
B*0702	**P**		**LMF***WYAIV*
B*3501	**P**		**LMFWY***IVA*
B51	**P**		**LIVF***WYAM*
B*5301	**P**		**IMFWY***ALV*
B*5401	**P**		**ATIV***LMFWY*

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

Table 2

SUPERMOTIFS		POSITION 1	2	3	4	5	6	7	8	C-terminus
A1			1° Anchor TIL*VMS*							1° Anchor FWY
A2			1° Anchor LIVM*ATQ*							1° Anchor LIVMAT
A3	preferred		1° Anchor VSMA*TLI*	YFW (4/5)			YFW (3/5)	YFW (4/5)	P (4/5)	1°Anchor RK
	deleterious	DE (3/5); P (5/5)		DE (4/5)						
A24			1° Anchor YF*WIVLM T*							1° Anchor F*IYWLM*
B7	preferred	FWY (5/5) LIVM (3/5)	1°Anchor P	FWY (4/5)					FWY (3/5)	1°Anchor VILF*MWYA*
	deleterious	DE (3/5); P(5/5); G(4/5); A(3/5); QN (3/5)				DE (3/5)	G (4/5)	QN (4/5)	DE (4/5)	
B27			1° Anchor RHK							1° Anchor FYL*WMIVA*
B44			1° Anchor E*D*							1° Anchor FWYLIMVA
B58			1° Anchor ATS							1° Anchor FWY*LIVMA*
B62			1° Anchor QL*IVMP*							1° Anchor FWY*MIVLA*

MOTIFS

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
A1 9-mer	preferred	GFYW	1°Anchor STM	DEA	YFW		P	DEQN	YFW	1°Anchor Y
	deleterious	DE		RHKLIVM P	A	G	A			

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
A1 9-mer	preferred	GRHK	ASTCLIV M	1°Anchor DE*AS*	GSTC		ASTC	LIVM	DE	1°Anchor Y
	deleterious	A	RHKDEPY FW		DE	PQN	RHK	PG	GP	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 10-mer	peferred	YFW	1°Anchor STM	DEAQN	A	YFWQN		PASTC	GDE	P	1°Anchor Y
	deleterious	GP		RHKGLVM	DE	RHK	QNA	RHKYFW	RHK	A	
A1 10-mer	preferred	YFW	STCLIVM	1°Anchor DE*AS*	A	YFW		PG	G	YFW	1°Anchor Y
	deleterious	RHK	RHKDEPYFW			P	G		PRHK	QN	
A2.1 9-mer	preferred	YFW	1°Anchor LM*IVQAT*	YFW	STC	YFW		A	P	1°Anchor V*LIMAT*	
	deleterious	DEP		DERKH			RKH	DERKH			
A2.1 10-mer	preferred	AYFW	1°Anchor LM*IVQAT*	LVIM	G		G		FYWLVIM		1°Anchor V*LIMAT*
	deleterious	DEP		DE	RKHA	P		RKH	DERKH	RKH	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3	preferred	RHK	1°Anchor LMVISAT *FCGD*	YFW	PRHKYFW	A	YFW		P	1°Anchor KYR*HFA*	
	deleterious	DEP		DE							
A11	preferred	A	1°Anchor VTLMISA *GNCDF*	YFW	YFW	A	YFW	YFW	P	1°Anchor K*RYH*	
	deleterious	DEP						A	G		
A24 9-mer	preferred	YFWRHK	1°Anchor YFW*M*		STC			YFW	YFW	1°Anchor FLIW	
	deleterious	DEG		DE	G	QNP	DERHK	G	AQN		
A24 10-mer	preferred		1°Anchor YFW*M*		P	YFWP		P			1°Anchor FLIW
	deleterious			GDE	QN	RHK	DE	A	QN	DEA	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3101	preferred	RHK	1°Anchor MVT*ALIS*	YFW	P		YFW	YFW	AP	C-terminus 1°Anchor R*K*	
	deleterious	DEP		DE		ADE	DE	DE	DE		
A3301	preferred		1°Anchor MVALF*IS T*	YFW				AYFW		1°Anchor RK	
	deleterious	GP		DE							
A6801	preferred	YFWSTC	1°Anchor AVT*MSLI*			YFWLIV M		YFW	P	1°Anchor RK	
	deleterious	GP		DEG		RHK			A		
B0702	preferred	RHKFWY	1°Anchor P	RHK		RHK	RHK	RHK	PA	1°Anchor LMF*WYAIV*	
	deleterious	DEQNP		DEP	DE	DE	GDE	QN	DE		
B3501	preferred	FWYLIVM	1°Anchor P	FWY				FWY		1°Anchor LMFWY*IVA*	
	deleterious	AGP				G	G				

		POSITION								
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
B51	preferred	LIVMFWY	1°Anchor P	FWY	STC	FWY		G	FWY	1°Anchor LIVF*WYAM*	
	deleterious	AGPDERHKSTC				DE	G	DEQN	GDE		
B5301	preferred	LIVMFWY	1°Anchor P	FWY	STC	FWY		LIVMFWY	FWY	1°Anchor IMFWY*ALV*	
	deleterious	AGPQN					G	RHKQN	DE		
B5401	preferred	FWY	1°Anchor P	FWYLIVM		LIVM		ALIVM	FWYAP	1°Anchor ATIV*LMFW Y*	
	deleterious	GPQNDE		GDESTC		RHKDE	DE	QNDGE	DE		

Italicized residues indicate less preferred or "tolerated" residues.
**The information in Table II is specific for 9-mers unless otherwise specified.

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Table 3

		POSITION								
MOTIFS		1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred	FMY*LIVW*	M	T		I	VST*CPALIM*	MH		MH
	deleterious				W			R		WDE
DR1	preferred	MF*LIVWY*			PAMQ		VMAT*SPLIC*	M		AVM
	deleterious		C	CH	FD	CWD		GDE	D	
DR7	preferred	MF*LIVWY*	M	W	A		IVMSA*CTPL*	M		IV
	deleterious		C		G			GRD	N	G
DR Supermotif		MF*LIVWY*					VMSTA*CPLI*			

DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH

Italicized residues indicate less preferred or "tolerated" residues.

TABLE 4. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
a. Individual Supertypes						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
b. Combined Supertypes						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

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Table 5

HLA-supertype	Allelle-specific HLA-supertype members	
	Verified[a]	Predicted[b]
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

a. Verified alleles include alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.

b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table 6a

B pocket residues defining an HLA-A1 supertype.

Antigen	Allele(s)	Motif Position 2	Reference(s)	B pocket residues 9	45	66	67	70
A1	A*0101	TS	14-16, 21, 62	F	M	N	M	H
A25	A*2501	TIM	18, 19	Y	M	N	V	H
A26	A*2601	VITLF	17	Y	M	N	V	H
A26	A*2602	VITLF	17	Y	M	N	V	H
A32	A*3201	I	20	F	M	N	V	H
A24	A*2402	YF	21-22, 24	S	M	K	V	H
B7	B*0702	P	1-2, 22, 64-65	Y	E	I	Y	Q
B27	B*2702	R	35	H	E	I	C	K
B44	B*4402	E	42-44	Y	K	I	S	N
B57	B*5701	ATS	33	Y	M	N	M	S
B62	B*1501	QL	33, 46, 49	Y	M	I	S	N
A1	A*0102	unknown		S	M	N	M	H
A26	A*2604	unknown	A*2501/A*2601	Y	M	N	V	H
A36	A*3601	unknown	A*0101	F	M	N	M	H
A43	A*4301	unknown	A*2501/A*2601	Y	M	N	V	H
A80	A*8001	unknown	A*3201	F	M	N	V	H

Table 6b

F pocket residues defining an HLA-A1 supertype.

Antigen	Allele(s)	Motif C-terminus	Reference(s)	F pocket residues 77	80	81	116
A1	A*0101	Y	14-16, 21, 62	N	T	L	D
A25	A*2501	W	18, 19	S	I	A	D
A26	A*2601	YF	17	N	T	L	D
A26	A*2602	YF	17	N	T	L	N
A32	A*3201	W	20	S	I	A	D
A2	A*0201	LIVMAT	1, 51-52	D	T	L	Y
A3	A*0301	RK	4, 21-22, 61	D	T	L	D
B7	B*0702	LIVMAFWY	1-2, 22, 64-65	S	N	L	Y
A1	A*0102	unknown	A*0101	N	T	L	D
A26	A*2604	unknown	A*0101	N	T	L	D
A36	A*3601	unknown	A*0101	N	T	L	D
A43	A*4301	unknown	A*0101	N	T	L	D
A80	A*8001	unknown	A*0101	N	T	L	D

Table 6c

Phenotypic frequency of the HLA-A1 supertype.

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
A1	A*0101-02	A*0101	A*0102	28.6	10.1	1.4	9.2	10.1	11.9
A25	A*2501	A*2501	-	6.1	1.6	0.0	0.8	4.2	2.5
A26	A*2601-04	A*2601-02	A*2604	7.3	3.2	20.4	3.8	6.7	8.3
A32	A*3201	A*3201	-	9.6	1.6	0.2	1.2	6.7	3.9
A36	A*3601	-	A*3601	0.8	5.3	0.0	0.4	0.6	1.4
A43	A*4301	-	A*4301	0.0	0.2	0.2	0.0	0.0	0.1
A80	A*8001	-	A*8001	-	-	-	-	-	-
Total coverage: defined				47.1	16.1	21.8	14.7	26.3	25.2
Total coverage: defined and predicted				47.6	21.1	22.0	15.1	26.8	26.5

Table 7a

B pocket residues defining an HLA-A24 supertype.

Antigen	Allele(s)	Motif Position 2	Reference(s)	B pocket residues 9	45	66	67	70
A23	A*2301	IY	25, 26	S	M	K	V	H
A24	A*2402	YF	21-22, 24	S	M	K	V	H
A30	A*3001	YFVLMIT	23	S	M	N	V	Q
A26	A*2601	VITLF	17	Y	M	V	H	H
A31	A*3101	LIVMAST	4, 62	T	M	N	V	H
B27	B*2702	R	35	H	E	I	C	K
B44	B*4402	E	42-44	Y	K	I	S	N
B57	B*5701	ATS	33	Y	M	N	M	S
B62	B*1501	QL	33, 46, 49	Y	M	I	S	N
B7	B*0702	P	1-2, 22, 64-65	Y	E	I	Y	Q
A24	A*2403	unknown	A*2402	S	M	K	V	H
A24	A*2404	unknown	A*2402	S	M	K	V	H
A30	A*3002-03	unknown		S	M	N	V	H

Table 7b

F pocket residues defining an HLA-A24 supertype.

Antigen	Allele(s)	Motif C-terminus	Reference(s)	F pocket residues 77	80	81	116
A23	A*2301	WI	25, 26	N	I	A	Y
A24	A*2402	FLI	21-22, 24	N	I	A	Y
A30	A*3001	LYFM	23	D	T	L	H
A2	A*0201	LIVMAT	1, 51-58	D	T	L	Y
A3	A*0301	RK	4, 21-22, 61	D	T	L	D
B7	B*0702	LIVMAFWY	1-2, 22, 64-65	S	N	L	Y
A24	A*2403	unknown	A*2402	N	I	A	Y
A24	A*2404	unknown		N	T	L	Y
A30	A*3002-03	unknown		N	T	L	H

Table 7c

Phenotypic frequency of the HLA-A24 supertype.

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
A23	A*2301	A*2301	-	3.2	14.3	0.0	1.6	5.5	4.9
A24	A*2402-04	A*2402	A*2403-04	16.8	8.8	58.1	32.9	26.7	28.7
A30	A*3001-03	A*3001	A*3002-03	4.7	18.8	0.8	7.3	8.4	8.0
Total coverage: defined and predicted				23.9	38.9	58.6	40.1	38.3	40.0

Table 8a

B pocket residues defining an HLA-B27 supertype.

Antigen	Allele(s)	Motif Position 2	Reference(s)	B pocket residues 9	45	66	67	70
B14	B*1401-02	RK	34	Y	E	I	C	N
B70	B*1509	H	33	Y	E	I	C	N
B27	B*2702	R	35	H	E	I	C	K
B27	B*2703	R	36, 40	H	E	I	C	K
B27	B*2704	R	36	H	E	I	C	K
B27	B*2705	R	35, 37	H	E	I	C	K
B27	B*2706	R	36	H	E	I	C	K
B38	B*3801-02	H	38	Y	E	I	C	N
B3901	B*3901	RH	38	Y	E	I	C	N
B3902	B*3902	KQ	38	Y	E	I	S	N
B73	B*7301	R	39	H	E	I	C	K
A1	A*0101	TS	14-16, 21, 62	F	M	N	M	H
A2	A*0201	LIVMAT	1, 51-58	F	M	K	V	H
A24	A*2402	YF	21-22, 24	S	M	K	V	H
B7	B*0702	P	1-2, 22, 64-65	Y	E	I	Y	Q
B44	B*4402	E	42-44	Y	K	I	S	N
B57	B*5701	ATS	33	Y	M	N	M	S
B62	B*1501	QL	33, 46, 49	Y	M	I	S	N
B27	B*2701	unknown	B*2702	H	E	I	C	K
B27	B*2707	unknown	B*2702	H	E	I	C	K
B27	B*2708	unknown	B*2702	H	E	I	C	K
B39	B*3903-05	unknown	B*3901	Y	E	I	C	N
B48	B*4801	unknown	B*3902	Y	E	I	S	N
B48	B*4802	unknown	B*3902	Y	E	I	S	N
B71	B*1510	unknown	B*1401 etc.	Y	E	I	C	N
B71	B*1518	unknown	B*1401 etc.	Y	E	I	C	N
B72	B*1503	unknown	B*3902	Y	E	I	S	N

Table 8b

F pocket residues defining an HLA-B27 supertype.

Antigen	Allele(s)	Motif C-terminus	Reference(s)	F pocket residues 77	80	81	116
B14	B*1401-02	L	34	S	N	L	F
B70	B*1509	LFM	33	S	N	L	Y
B27	B*2702	FWYILM	35	N	I	A	D
B27	B*2703	YF	36, 40	D	T	L	D
B27	B*2704	YLF	36	S	T	L	D
B27	B*2705	YLFMIRHK	35, 37	D	T	L	D
B27	B*2706	L	36	S	T	L	Y
B38	B*3801-02	FL	38	N	I	A	F
B3901	B*3901, 03-05	L	38	S	N	L	F
B3902	B*3902	L	38	S	N	L	F
B73	B*7301	-	39	G	N	L	F
B27	B*2701	unknown		N	T	A	D
B27	B*2707	unknown		D	T	L	Y
B27	B*2708	unknown		S	N	L	D
B39	B*3903-05	unknown	B*3901-02	S	N	L	F
B48	B*4801	unknown	B*1509	S	N	L	Y
B48	B*4802	unknown		S	N	L	S
B71	B*1510	unknown	B*1509	S	N	L	Y
B71	B*1518	unknown		S	N	L	S
B72	B*1503	unknown		S	N	L	S

Table 8c

Phenotypic frequency of the HLA-B27 supertype.

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
B14	B*1401-02	B*1401-02	-	7.6	6.3	0.4	0.8	12.4	5.5
B27	B*2701-08	B*2702-06	B*2701, 07, 08	7.5	2.6	0.8	3.4	4.9	3.8
B38	B*3801-02	B*3801-02	-	7.6	0.2	0.8	3.4	3.6	3.1
B39	B*3901-04	B*3901-02	B*3903-04	3.6	1.6	8.4	5.1	11.8	6.1
B70(B71/B72)	B*1503, 09, 10, 18	B*1509	B*1503, 10, 18	3.2	15.9	3.2	1.6	4.9	5.8
B73	B*7301	B*7301	-	0.8	0.6	0.0	0.0	0.6	0.4
B48	B48	-	B*4801-02	0.4	0.6	5.5	5.5	4.5	3.3
Total coverage: defined				28.4	26.1	13.3	13.9	35.3	23.4
Total coverage: defined and predicted				28.8	26.6	18.5	19.0	38.9	26.3

Table 9a

B pocket residues defining an HLA-B44 supertype.

Antigen	Allele(s)	Motif Position 2	Reference(s)	B pocket residues 9	45	63	66	67	70	99
B18	B*1801-02	E	47	H	T	N				
B37	B*3701	DE	41	H	T	E				
B44	B*4402-04	E	42-44	Y	K	E				S
B60	B*4001	E	45	H	K	E				
B61	B*4002, 06	E	46	H	K	E				
A2	A*0201	LIVMAT	1, 51-58	F	M	E	K	V	H	
A24	A*2402	YF	21-22, 24	S	M	E	K	V	H	F
B7	B*0702	P	1-2, 22, 64-65	Y	E	N		Y	Q	
B27	B*2702	R	35	H	E	E		C	K	
B57	B*5701	ATS	33	Y	M	E	N	M	S	
B62	B*1501	QL	33, 46, 49	Y	M	E				
B41	B*4101	unknown	B*4402	H	K	E				
B45	B*4501	unknown	B*4402	H	K	E				
B47	B*4701	unknown		Y	K	E				F
B49	B*4901	unknown	B*4402	H	K	E				
B50	B*5001	unknown	B*4402	H	K	E				

Table 9b

F pocket residues defining an HLA-B44 supertype.

Antigen	Allele(s)	Motif C-terminus	Reference(s)	F pocket residues 77	80	81	116
B18	B*1801-02	Y	47	S	N	L	S
B37	B*3701	FMLI	41	D	T	L	F
B44	B*4402-04	FY	42-44	N	T	A	D
B60	B*4001	LWMAV	45	S	N	L	Y
B61	B*4002, 06	LWMAV	46	S	N	L	Y
B41	B*4101	unknown	B*4001	S	N	L	Y
B45	B*4501	unknown		S	N	L	L
B47	B*4701	unknown		D	T	L	D
B49	B*4901	unknown		N	I	A	L
B50	B*5001	unknown		S	N	I	L

Table 9c

Phenotypic frequency of the HLA-B44 supertype.

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
B18	B*1801-02	B*1801-02	-	9.2	6.9	0.0	2.2	7.6	5.2
B37	B*3701	B*3701	-	4.4	2.2	1.6	3.8	1.2	2.6
B44	B*4402-04	B*4402-04	-	19.7	10.5	14.3	6.7	17.4	13.7
B60	B*4001	B*4001	-	8.2	2.2	10.7	20.1	6.1	9.5
B61	B*4002, 06-07	B*4002, 06	-	5.9	0.2	20.3	9.6	10.1	9.2
B41	B*4101	-	B*4101	2.0	4.2	0.0	0.0	6.7	2.6
B45	B*4501	-	B*4501	1.2	7.1	0.0	0.8	3.6	2.5
B47	B*4701	-	B*4701	1.2	0.0	0.2	0.0	1.2	0.5
B49	B*4901	-	B*4901	4.4	3.6	0.0	0.4	4.9	2.7
B50	B*5001	-	B*5001	4.4	2.2	0.0	3.0	5.5	3.0
Total coverage: defined				43.0	21.2	42.9	39.1	39.0	37.0
Total coverage: defined and predicted				52.6	35.8	43.1	42.4	55.1	45.8

Table 10a

B pocket residues defining an HLA-B58 supertype.

Antigen	Allele(s)	Avg population coverage (antigen)	Motif Position 2	Reference(s)	B pocket residues				
					9	45	66	67	70
B57	B*5701	3.9 (B57)	ATS	33	Y	M	N	M	S
B57	B*5702	* (B57)	ATS	33	Y	M	N	M	S
B58/B17	B*5801-02	5.4 (B58)	AST	33, 46	Y	T	N	M	S
B63	B*1516	1.4 (B63)	STA	33	Y	M	N	M	S
B63	B*1517	* (B63)	TS	33	Y	M	N	M	S
A1	A*0101	11.9 (A1)	TS	14-16, 21, 62	F	M	N	M	H
A3	A*0301-02	12.0 (A3)	LIVMAST	4, 21-22, 61	F	M	N	V	Q
A24	A*2402-03	28.7 (A24)	YF	21-22, 24	S	M	K	V	H
B7	B*0702, 04-05	12.3 (B7)	P	1-2, 22, 64-65	Y	E	I	Y	Q
B27	B*2702	3.8 (B27)	R	35	H	E	I	C	K
B44	B*4402-04	13.7 (B44)	E	42-44	Y	K	I	S	N
B62	B*1501, 04-05, 07, 20	9.3 (B62)	QL	33, 46, 49	Y	M	I	S	N

Table 10b

F pocket residues defining an HLA-B58 supertype.

Antigen	Allele(s)	Avg population coverage (antigen)	Motif C-terminus	Reference(s)	F pocket residues			
					77	80	81	116
B57	B*5701	3.9 (B57)	FWY	33	N	I	A	S
B57	B*5702	* (B57)	FWY	33	N	I	A	Y
B58/B17	B*5801-02	5.4 (B58)	FW	33, 46	N	I	A	S
B63	B*1516	1.4 (B63)	IVFY	33	N	I	A	S
B63	B*1517	* (B63)	YFLI	33	N	I	A	D
A2	A*0201	8.5 (A31)	LIVMAT	1, 51-58	D	T	L	Y
A3	A*0301	8.5 (A31)	RK	4, 21-22, 61	D	T	L	D
B7	B*0702	12.3 (B7)	LIVMAFWY	1-2, 22, 64-65	S	N	L	Y

Table 10c

Phenotypic frequency of the HLA-B58 supertype.

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
B57	B*5701-02	B*5701-02	-	7.3	7.6	0.0	2.0	2.4	3.9
B58/B17	B*5801-02	B*5801-02	-	2.0	13.7	1.4	6.7	3.0	5.4
B63	B*1516, 17	B*1516, 17	-	0.8	4.9	0.2	0.4	0.6	1.4
Total coverage: defined and predicted				10.0	25.1	1.6	9.0	5.9	10.3

Table 11a

B pocket residues defining an HLA-B62 supertype.

Antigen	Allele(s)	Motif Position 2	Reference(s)	B pocket residues 9	45	66	67	70
B52	B*5201	Q	48	Y	T	L	S	N
B62	B*1501	QL	33, 46, 49	Y	M	T	S	N
B75	B*1502	QLVP	33	Y	M	L	S	N
B77	B*1513	LIQVPM	33	Y	M	T	S	N
A2	A*0201	LIVMAT	1, 51-58	F	M	K	V	H
A24	A*2402-03	YF	21-22, 24	S	M	K	V	H
B7	B*0702, 04-05	P	1-2, 22, 64-65	Y	E	T	Y	Q
B27	B*2702	R	35	H	E	T	C	K
B44	B*4402-04	E	42-44	Y	K	T	S	N
B57	B*5701	ATS	33	Y	M	N	M	S
B13	B*1301-02	unknown	B*1501	Y	M	I	S	N
B62	B*1504-07, 15, 20	unknown	B*1501	Y	M	I	S	N
B75	B*1521	unknown		Y	M	I	C	N
B76	B*1512, 14, 19	unknown	B*1501	Y	M	I	S	N

Table 11b

F pocket residues defining an HLA-B62 supertype.

Antigen	Allele(s)	Motif C-terminus	Reference(s)	F pocket residues 77	80	81	116
B62	B*1501	FY	33, 46, 49	S	N	L	S
B75	B*1502	FYM	33	S	N	L	S
B77	B*1513	W	33	N	I	A	S
B52	B*5201	IV	48	N	I	A	Y
A2	A*0201	LIVMAT	1, 51-58	D	T	L	Y
A3	A*0301	RK	4, 21-22, 61	D	T	L	D
A24	A*2402	FLI	21-22, 24	N	I	A	Y
B7	B*0702	LIVMAFWY	1-2, 22, 64-65	S	N	L	Y
B44	B*4402	FY	42-44	N	T	A	D
B13	B*1301-02	unknown		N	T	A	L
B62	B*1504-07, 15, 20	unknown	B*1501	S	N	L	S
B75	B*1521	unknown	B*1501	S	N	L	S
B76	B*1512, 14, 19	unknown	B*1501	S	N	L	S

Table 11c

Phenotypic frequency of the HLA-B62 supertype.

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
B52	B*5201	B*5201	-	2.4	1.4	20.3	4.9	5.9	7.0
B62	B*1501, 04-08, 15, 20	B*1501	B*150 -07, 15, 20	10.3	2.6	16.3	14.3	3.0	9.3
B75	B*1502, 21	B*1502	B*1521	0.0	0.8	2.2	7.3	2.4	2.5
B77	B*1513	B*1513	-	-	-	-	-	-	-
B13	B*1301-02	-	B*1301-02	5.9	1.6	3.4	15.7	3.0	5.9
B76	B*1512, 14, 19	-	B*1512, 14, 19	0.4	0.0	0.0	0.0	0.0	0.1
Total coverage: defined				12.6	4.8	36.5	25.4	11.1	18.1
Total coverage: defined and predicted				18.4	6.3	39.3	38.9	13.9	23.4

Table 12

Assays necessary to evaluate binding specificities of the putative HLA-A1-, A24-, and B44-supertypes.

Supertype	Antigen	Allele	Source (cell line)[a]	Comments
A1	A1	A*0101	Steinlin[b]	assay established
	A25	A*2501	D0208915, BM92	
	A26	A*2601	QBL, MGAR, YAR	
	A26	A*2602	TEM	
	A32	A*3201	JBUSH, WT47	
A24	A23	A*2301	WT51	
	A24	A*2402	KT3, KAS116, TISI	A*24 (KT3) assay established
	A30	A*3001	LBUF (LBF)	
B44	B18	B*1801-02	D0208915, DUCAF, QBL, BM16, JVM, EJ32B	
	B37	B*3701	KAS011	
	B44	B*4402-04	WT47,AWELLS, HOR, EK, MOU, SP0010	B*4403 (PITOUT) assay established
	B60	B*4001	LB, HHD, DKB, MT14B,EMJ, PE117	
	B61	B*4002, 06	SWEIG	

a. Cell lines indicate are EBV transformed and homozygous for the antigen indicated.
b. Cell lines indicated in bold font are already available in house.

WHAT IS CLAIMED IS

1. A method for evaluating properties of a peptide comprising an epitope that comprises an HLA-A supermotif, said peptide comprising an epitope consisting of about 8-11 residues that binds to at least two HLA molecules, and when bound to either of the at least two HLA molecules induces a cytotoxic T cell response, or when bound to an HLA molecule of a HLA-A supertype binds at an affinity of an IC_{50} of less than about 500 nM, with a *proviso* that the HLA-A supermotif is neither an HLA-A2 supermotif nor N HLA-A3 supermotif, said method comprising step of:

interacting the peptide with at least two HLA molecules, with a *proviso* that the at least two HLA molecules are not members of an HLA-A2 supertype or are not members of an HLA-A3 supertype, and when bound to either of the at least two HLA molecules induces a cytotoxic T cell response; or,

associating the peptide with at least one HLA-A molecule, with a *proviso* that the at least one HLA-A molecule is neither a member of the HLA-A2 supertype nor of the HLA-A3 supertype, whereby the peptide binds at an affinity of an IC_{50} of less than about 500 nM.

2. The method of claim 1 comprising making an immunogenic peptide that bears an HLA-A1 supermotif and inducing an immune response, said peptide comprising an epitope consisting of about 8-11 residues that binds to multiple HLA molecules, and when bound to such an HLA molecule induces a cytotoxic T cell response, said method comprising steps of:

providing an amino acid sequence of an antigen of interest or a peptide fragment thereof, having an amino terminus and a carboxyl terminus;

identifying a putative T cell epitope within said amino acid sequence or a peptide fragment thereof, whereby said putative epitope comprises the structural HLA-A1 supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid residue at position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of T, S, L, I, V, and M, and a residue selected from the group consisting of Y, F, and W as a carboxyl-terminal amino acid residue of the epitope;

obtaining a peptide fragment derived from the antigen that comprises the HLA-A1 structural supermotif;

testing a complex of said peptide fragment and a first HLA-A1 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope;

testing a complex of said peptide fragment and at least a second HLA-A1 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope; and,

selecting said peptide fragment comprising the HLA-A1 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope when in complex with the first HLA-A1 supertype molecule and when in complex with the at least a second HLA-A1 supertype molecule.

3. The method of claim 2, wherein the peptide fragment has 8, 9, 10 or 11 residues.

4. The method of claim 2, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

5. The method of claim 2, further comprising a step of determining binding affinity of the peptide fragment for an HLA molecule selected from the group consisting of HLA-A*0101, HLA-A*2501, HLA-A*2601, HLA-A*2602, HLA-A*3201, HLA-A*0102, HLA-A*2604, HLA-A*3601, HLA-A*4301, and HLA-A*8001.

6. The method of claim 5, further comprising a step of identifying the peptide fragment which has a binding affinity of an IC_{50} of less than about 500 nM for the HLA molecule.

7. The method of claim 2, wherein the obtaining step comprises expressing in a cell a recombinant nucleic acid molecule that encodes the peptide fragment.

8. The method of claim 7, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the peptide fragment and one or more additional peptides; with a *proviso* that neither the peptide fragment, the one

or more additional peptides, nor any combination of the peptide fragment and the one or more additional peptides comprise an entire native antigen.

9. The method of claim 2, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

10. The method of claim 2, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vitro*.

11. The method of claim 2, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vivo*.

12. The method of claim 2, wherein the providing step comprises providing an amino acid sequence of an antigen of interest which is a cancer-associated antigen.

13. The method of claim 12, wherein the providing step comprises providing an amino acid sequence from an antigen that is HER2/neu, p53, MAGE, or prostate antigen.

14. The method of claim 2, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

15. The method of claim 14, wherein the providing step comprises providing an amino acid sequence from an antigen that is an HBV, HCV, HIV, HPV, or malaria antigen.

16. The method of claim 1 comprising making an HLA-A1 supermotif peptide that binds to an HLA molecule at an IC50 of less than about 500 nM, the method comprising steps of:

(a) providing an amino acid sequence of an antigen of interest;

(b) identifying within said sequence a putative T cell epitope from the provided amino acid sequence, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-A1 structural supermotif associated with peptide binding to multiple HLA-A1 supertype molecules, said structural motif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of T, S, L, I, V, and M, and a second amino acid anchor residue selected from the group consisting of Y, F, and W as carboxyl-terminal amino acid residue of the epitope;

(c) obtaining a peptide fragment of said antigen of interest that comprises the HLA-A1 structural supermotif;

(d) contacting said peptide fragment of step (c) with a first HLA molecule selected from the group consisting of HLA-A*0101, A*2501, A*2601, A*2602, A*3201, A*0102, A*2604, A*3601, A*4301, and A*8001;

(e) contacting said peptide fragment of step (c) with a second HLA molecule selected from the group consisting of HLA-A*0101, A*2501, A*2601, A*2602, A*3201, A*0102, A*2604, A*3601, A*4301, and A*8001, wherein the second HLA molecule is different from the first HLA molecule;

(f) determining binding affinity of the peptide fragment for the first and the second HLA molecule; and,

(g) selecting the peptide fragment that comprise an HLA-A1 structural supermotif that binds to at least the first or at least the second HLA molecule at a binding affinity of an IC_{50} of less than about 500 nM.

17. The method of claim 16, further comprising a step of:

(h) contacting an HLA-A*0101, HLA-A*2501, HLA-A*2601, HLA-A*2602, HLA-A*3201, HLA-A*0102, HLA-A*2604, HLA-A*3601, HLA-A*4301, or HLA-A*8001 restricted cytotoxic T lymphocyte with a complex of the peptide of step (g) and an HLA-A*0101, HLA-A*2501, HLA-A*2601, HLA-A*2602, HLA-A*3201, HLA-A*0102, HLA-A*2604, HLA-A*3601, HLA-A*4301, or HLA-A*8001 molecule, respectively.

18. The method of claim 16, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vitro*.

19. The method of claim 16, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vivo*.

20. The method of claim 1 comprising inducing an immune response with a peptide comprising an epitope consisting of about 8-11 residues that will bind to multiple HLA-A1 supertype molecules and induce an HLA-restricted cytotoxic T cell response, said method comprising steps of:

providing a peptide comprising a putative T cell epitope, said putative epitope comprising an HLA-A1 structural supermotif associated with peptide binding to multiple HLA-A1 supertype molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of T, S, L, I, V, and M, and a second amino acid anchor residue selected from the group consisting of Y, F, and W as the carboxyl-terminal amino acid residue of the epitope;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with a first HLA-A1 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with at least a further HLA-A1 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the at least a further complex.

21. The method of claim 20, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA- HLA-A*0101, HLA-A*2501, HLA-A*2601, HLA-A*2602, HLA-A*3201, HLA-A*0102, HLA-A*2604, HLA-A*3601, HLA-A*4301, and HLA-A*8001 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

22. The method of claim 20, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

23. The method of claim 22, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof which comprises an IC_{50} of less than about 50 nM for at least one HLA molecule.

24. The method of claim 22, wherein the at least one HLA molecule is selected from the group consisting of: HLA-A*0101, HLA-A*2501, HLA-A*2601, HLA-A*2602, HLA-A*3201, HLA-A*0102, HLA-A*2604, HLA-A*3601, HLA-A*4301, and HLA-A*8001 molecules.

25. The method of claim 20, wherein the providing step comprises providing the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

26. The method of claim 20, wherein said provided peptide is connected to another molecule to create a compound, with a *proviso* that neither said peptide, said another molecule, nor said compound comprise an entire native antigen.

27. The method of claim 26, wherein the another molecule is a lipid.

28. The method of claim 26, wherein the another molecule is a T helper epitope.

29. The method of claim 26, wherein the another molecule is a cytotoxic T lymphocyte (CTL) epitope.

30. The method of claim 29, wherein the another molecule is the peptide.

31. The method of claim 26, wherein the another molecule is a carrier molecule.

32. The method of claim 20, wherein the providing step comprises providing a peptide from a cancer-associated antigen.

33. The method of claim 20, wherein the providing step comprises providing a peptide from an antigen that is derived from a pathogenic agent.

34. The method of claim 20, wherein the providing step comprises providing a peptide of more than 11 residues.

35. The method of claim 20, wherein the providing step comprises providing a peptide of 8, 9, 10 or 11 residues.

36. The method of claim 20, wherein the providing step comprises isolation of the one or more peptides from a natural source or comprises chemical synthesis of the peptide.

37. The method of claim 20, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide.

38. The method of claim 37, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide and at least one additional peptide, with a proviso that neither an additional peptide nor a combination of the peptide and an additional peptide comprise an entire native antigen.

39. The method of claim 20, wherein the first complexing step and/or the further complexing step occurs *in vitro*.

40. The method of claim 20, wherein the first complexing step and/or the further complexing step occurs *in vivo*.

41. The method of claim 20, wherein the first contacting step and/or the further contacting occurs *in vitro*.

42. The method of claim 20, wherein the first contacting step and/or the further contacting occurs *in vivo*.

43. The method of claim 1 comprising inducing an immune response, said method comprising steps of:

providing a nucleic acid encoding a peptide comprising a putative HLA epitope of about 8-11 amino acids in length, said epitope comprising an HLA-A1 structural supermotif associated with binding to multiple HLA molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of T, S, L, I, V, and M, and a second amino acid anchor residue selected from the group consisting of Y, F, and W as the carboxyl-terminal amino acid residue of the epitope;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with a first HLA-A1 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with at least a further HLA-A1 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the further complex.

44. The method of claim 43, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA- HLA-A*0101, HLA-A*2501, HLA-A*2601, HLA-A*2602, HLA-A*3201, HLA-A*0102, HLA-A*2604, HLA-A*3601, HLA-A*4301, and HLA-A*8001 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

45. The method of claim 43, wherein the providing step comprises providing a nucleic acid encoding the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

46. The method of claim 43, wherein the providing step comprises providing a a nucleic acid encoding a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

47. The method of claim 1 comprising making an immunogenic peptide that bears an HLA-A24 supermotif and inducing an immune response, said peptide comprising an epitope consisting of about 8-11 residues that binds to multiple HLA molecules, and when bound to such an HLA molecule induces a cytotoxic T cell response, said method comprising steps of:

providing an amino acid sequence of an antigen of interest or a peptide fragment thereof, having an amino terminus and a carboxyl terminus;

identifying a putative T cell epitope within said amino acid sequence or a peptide fragment thereof, whereby said putative epitope comprises the structural HLA-A24 supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid residue at position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of Y, F, W, I, V, L, M, and T, and a residue selected from the group consisting of F, I, Y, W, L, and M as a carboxyl-terminal amino acid residue of the epitope;

obtaining a peptide fragment derived from the antigen that comprises the HLA-A24 structural supermotif;

testing a complex of said peptide fragment and a first HLA-A24 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope;

testing a complex of said peptide fragment and at least a second HLA-A24 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope; and,

selecting said peptide fragment comprising the HLA-A24 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope when in complex with the first HLA-A24 supertype molecule and when in complex with the at least a second HLA-A24 supertype molecule.

48. The method of claim 47, wherein the peptide fragment has 8, 9, 10 or 11 residues.

49. The method of claim 47, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

50. The method of claim 47, further comprising a step of determining binding affinity of the peptide fragment for an HLA molecule selected from the group consisting of HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, and HLA-A*3003.

51. The method of claim 50, further comprising a step of identifying the peptide fragment which has a binding affinity of an IC_{50} of less than about 500 nM for the HLA molecule.

52. The method of claim 47, wherein the obtaining step comprises expressing in a cell a recombinant nucleic acid molecule that encodes the peptide fragment.

53. The method of claim 52, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the peptide fragment and one or more additional peptides; with a *proviso* that neither the peptide fragment, the one or more additional peptides, nor any combination of the peptide fragment and the one or more additional peptides comprise an entire native antigen.

54. The method of claim 47, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

55. The method of claim 47, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vitro*.

56. The method of claim 47, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vivo*.

57. The method of claim 47, wherein the providing step comprises providing an amino acid sequence of an antigen of interest which is a cancer-associated antigen.

58. The method of claim 57, wherein the providing step comprises providing an amino acid sequence from an antigen that is HER2/neu, p53, MAGE, or prostate antigen.

59. The method of claim 47, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

60. The method of claim 59, wherein the providing step comprises providing an amino acid sequence from an antigen that is an HBV, HCV, HIV, HPV, or malaria antigen.

61. The method of claim 1, comprising making an HLA-A24 supermotif peptide that binds to an HLA molecule at an IC_{50} of less than about 500 nM, the method comprising steps of:

(a) providing an amino acid sequence of an antigen of interest;

(b) identifying within said sequence a putative T cell epitope from the provided amino acid sequence, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-A24 structural supermotif associated with peptide binding to multiple HLA-A24 supertype molecules, said structural motif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of Y, F, W, I, V, L, M, and T, and a second amino acid anchor residue selected from the group consisting of F, I, Y, W, L, and M as carboxyl-terminal amino acid residue of the epitope;

(c) obtaining a peptide fragment of said antigen of interest that comprises the HLA-A24 structural supermotif;

(d) contacting said peptide fragment of step (c) with a first HLA molecule selected from the group consisting of HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, and HLA-A*3003;

(e) contacting said peptide fragment of step (c) with a second HLA molecule selected from the group consisting of HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, and HLA-A*3003, wherein the second HLA molecule is different from the first HLA molecule;

(f) determining binding affinity of the peptide fragment for the first and the second HLA molecule; and,

(g) selecting the peptide fragment that comprise an HLA-A24 structural supermotif that binds to at least the first or at least the second HLA molecule at a binding affinity of an IC_{50} of less than about 500 nM.

62. The method of claim 61, further comprising a step of:

(h) contacting an HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, or HLA-A*3003 restricted cytotoxic T lymphocyte with a complex of the peptide of step (g) and an HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, or HLA-A*3003 molecule, respectively.

63. The method of claim 61, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vitro*.

64. The method of claim 61, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vivo*.

65. The method of claim 1 comprising of inducing an immune response with a peptide comprising an epitope consisting of about 8-11 residues that will bind to multiple HLA-A24 supertype molecules and induce an HLA-restricted cytotoxic T cell response, said method comprising steps of:

providing a peptide comprising a putative T cell epitope, said putative epitope comprising an HLA-A24 structural supermotif associated with peptide binding to multiple HLA-A24 supertype molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of Y, F, W, I, V, L, M, and T, and a second amino acid anchor residue selected from the group consisting of F, I, Y, W, L, and M as the carboxyl-terminal amino acid residue of the epitope;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with a first HLA-A24 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with at least a further HLA-A24 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the at least a further complex.

66. The method of claim 65, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, and HLA-A*3003 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

67. The method of claim 65, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

68. The method of claim 67, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof which comprises an IC_{50} of less than about 50 nM for at least one HLA molecule.

69. The method of claim 67, wherein the at least one HLA molecule is selected from the group consisting of: HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, and HLA-A*3003 molecules.

70. The method of claim 65, wherein the providing step comprises providing the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

71. The method of claim 65, wherein said provided peptide is connected to another molecule to create a compound, with a *proviso* that neither said peptide, said another molecule, nor said compound comprise an entire native antigen.

72. The method of claim 71, wherein the another molecule is a lipid.

73. The method of claim 71, wherein the another molecule is a T helper epitope.

74. The method of claim 71, wherein the another molecule is a cytotoxic T lymphocyte (CTL) epitope.

75. The method of claim 74, wherein the another molecule is the peptide.

76. The method of claim 71, wherein the another molecule is a carrier molecule.

77. The method of claim 65, wherein the providing step comprises providing a peptide from a cancer-associated antigen.

78. The method of claim 65, wherein the providing step comprises providing a peptide from an antigen that is derived from a pathogenic agent.

79. The method of claim 65, wherein the providing step comprises providing a peptide of more than 11 residues.

80. The method of claim 65, wherein the providing step comprises providing a peptide of 8, 9, 10 or 11 residues.

81. The method of claim 65, wherein the providing step comprises isolation of the one or more peptides from a natural source or comprises chemical synthesis of the peptide.

82. The method of claim 65, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide.

83. The method of claim 82, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide and at least one additional peptide, with a *proviso* that neither an additional peptide nor a combination of the peptide and an additional peptide comprise an entire native antigen.

84. The method of claim 65, wherein the first complexing step and/or the further complexing step occurs *in vitro*.

85. The method of claim 65, wherein the first complexing step and/or the further complexing step occurs *in vivo*.

86. The method of claim 65, wherein the first contacting step and/or the further contacting occurs *in vitro*.

87. The method of claim 65, wherein the first contacting step and/or the further contacting occurs *in vivo*.

88. The method of claim 1 comprising inducing an immune response, said method comprising steps of:

providing a nucleic acid encoding a peptide comprising a putative HLA epitope of about 8-11 amino acids in length, said epitope comprising an HLA-A24 structural supermotif associated with binding to multiple HLA molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of Y, F, W, I, V, L, M, and T, and a second amino acid anchor residue selected from the group consisting of F, I, Y, W, L, and M as the carboxyl-terminal amino acid residue of the epitope;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with a first HLA-A24 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with at least a further HLA-A24 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the further complex.

89. The method of claim 88, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-A*2301, HLA-A*2402, HLA-A*3001, HLA-A*2403, HLA-A*2404, HLA-A*3002, and HLA-A*3003 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

90. The method of claim 88, wherein the providing step comprises providing a nucleic acid encoding the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

91. The method of claim 88, wherein the providing step comprises providing a a nucleic acid encoding a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

92. A method for evaluating the properties of a peptide comprising an epitope that comprises an HLA-B supermotif, said epitope consisting of about 8-11 residues that bind to at least two HLA molecules, and when bound to either of the at least two HLA molecules induces a cytotoxic T cell response, or when bound to an HLA molecule of an HLA-B supertype binds at an affinity of an IC_{50} of less than about 500 nM, with a *proviso* that the HLA-B supermotif is not an HLA-B7 supermotif, said method comprising step of:

interacting the peptide with at least two HLA molecules, with a *proviso* that the at least two HLA molecules are not members of an HLA-B7 supertype, and when bound to either of the at least two HLA molecules induces a cytotoxic T cell response; or,

associating the peptide with at least one HLA-B molecule, with a *proviso* that the HLA-B molecule is not a member of the HLA-B7 supertype, whereby the peptide binds at an affinity of an IC_{50} of less than about 500 nM.

93. The method of claim 92 comprising making an immunogenic peptide that bears an HLA-B27 supermotif and inducing an immune response, said peptide comprising an epitope consisting of about 8-11 residues that binds to multiple HLA molecules, and when bound to such an HLA molecule induces a cytotoxic T cell response, said method comprising steps of:

providing an amino acid sequence of an antigen of interest or a peptide fragment thereof, having an amino terminus and a carboxyl terminus;

identifying a putative T cell epitope within said amino acid sequence or a peptide fragment thereof, whereby said putative epitope comprises the structural HLA-B27 supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid residue at position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of R, H, and K, and a residue selected from the group consisting of F, Y, L, W, M, I, V, and A as a carboxyl-terminal amino acid residue of the epitope;

obtaining a peptide fragment derived from the antigen that comprises the HLA-B27 structural supermotif;

testing a complex of said peptide fragment and a first HLA-B27 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope;

testing a complex of said peptide fragment and at least a second HLA-B27 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope; and,

selecting said peptide fragment comprising the HLA-B27 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope when in complex with the first HLA-B27 supertype molecule and when in complex with the at least a second HLA-B27 supertype molecule.

94. The method of claim 93, wherein the peptide fragment has 8, 9, 10 or 11 residues.

95. The method of claim 93, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

96. The method of claim 93, further comprising a step of determining binding affinity of the peptide fragment for an HLA molecule selected from the group consisting of HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, and HLA-B*1503.

97. The method of claim 96, further comprising a step of identifying the peptide fragment which has a binding affinity of an IC_{50} of less than about 500 nM for the HLA molecule.

98. The method of claim 93, wherein the obtaining step comprises expressing in a cell a recombinant nucleic acid molecule that encodes the peptide fragment.

99. The method of claim 98, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the peptide fragment and one or more additional peptides; with a *proviso* that neither the peptide fragment, the one or more additional peptides, nor any combination of the peptide fragment and the one or more additional peptides comprise an entire native antigen.

100. The method of claim 93, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

101. The method of claim 93, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vitro*.

102. The method of claim 93, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vivo*.

103. The method of claim 93, wherein the providing step comprises providing an amino acid sequence of an antigen of interest which is a cancer-associated antigen.

104. The method of claim 103, wherein the providing step comprises providing an amino acid sequence from an antigen that is HER2/neu, p53, MAGE, or prostate antigen.

105. The method of claim 93, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

106. The method of claim 105, wherein the providing step comprises providing an amino acid sequence from an antigen that is an HBV, HCV, HIV, HPV, or malaria antigen.

107. A method of claim 92 comprising making an HLA-B27 supermotif peptide that binds to an HLA molecule at an IC_{50} of less than about 500 nM, the method comprising steps of:

(a) providing an amino acid sequence of an antigen of interest;

(b) identifying within said sequence a putative T cell epitope from the provided amino acid sequence, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-B27 structural supermotif associated with peptide binding to multiple HLA-B27 supertype molecules, said structural motif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of R, H, and K, and a second amino acid anchor residue selected from the group consisting of F, Y, L, W, M, I, V, and A as carboxyl-terminal amino acid residue of the epitope;

(c) obtaining a peptide fragment of said antigen of interest that comprises the HLA-B27 structural supermotif;

(d) contacting said peptide fragment of step (c) with a first HLA molecule selected from the group consisting of HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801,

HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, and HLA-B*1503;

(e) contacting said peptide fragment of step (c) with a second HLA molecule selected from the group consisting of HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, and HLA-B*1503, wherein the second HLA molecule is different from the first HLA molecule;

(f) determining binding affinity of the peptide fragment for the first and the second HLA molecule; and,

(g) selecting the peptide fragment that comprise an HLA-B27 structural supermotif that binds to at least the first or at least the second HLA molecule at a binding affinity of an IC_{50} of less than about 500 nM.

108. The method of claim 107, further comprising a step of:

(h) contacting an HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, or HLA-B*1503 restricted cytotoxic T lymphocyte with a complex of the peptide of step (g) and an HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, or HLA-B*1503 molecule, respectively.

109. The method of claim 107, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vitro*.

110. The method of claim 107, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vivo*.

111. The method of claim 92 comprising inducing an immune response with a peptide comprising an epitope consisting of about 8-11 residues that will bind to multiple HLA-B27 supertype molecules and induce an HLA-restricted cytotoxic T cell response, said method comprising steps of:

providing a peptide comprising a putative T cell epitope, said putative epitope comprising an HLA-B27 structural supermotif associated with peptide binding to multiple HLA-B27 supertype molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of R, H, and K, and a second amino acid anchor residue selected from the group consisting of F, Y, L, W, M, I, V, and A as the carboxyl-terminal amino acid residue of the epitope;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with a first HLA-B27 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with at least a further HLA-B27 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the at least a further complex.

112. The method of claim 111, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, and HLA-B*1503 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

113. The method of claim 111, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

114. The method of claim 113, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof which comprises an IC_{50} of less than about 50 nM for at least one HLA molecule.

115. The method of claim 113, wherein the at least one HLA molecule is selected from the group consisting of: HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, and HLA-B*1503 molecules.

116. The method of claim 111, wherein the providing step comprises providing the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

117. The method of claim 111, wherein said provided peptide is connected to another molecule to create a compound, with a *proviso* that neither said peptide, said another molecule, nor said compound comprise an entire native antigen.

118. The method of claim 117, wherein the another molecule is a lipid.

119. The method of claim 117, wherein the another molecule is a T helper epitope.

120. The method of claim 117, wherein the another molecule is a cytotoxic T lymphocyte (CTL) epitope.

121. The method of claim 120, wherein the another molecule is the peptide.

122. The method of claim 117, wherein the another molecule is a carrier molecule.

123. The method of claim 111, wherein the providing step comprises providing a peptide from a cancer-associated antigen.

124. The method of claim 111, wherein the providing step comprises providing a peptide from an antigen that is derived from a pathogenic agent.

125. The method of claim 111, wherein the providing step comprises providing a peptide of more than 11 residues.

126. The method of claim 111, wherein the providing step comprises providing a peptide of 8, 9, 10 or 11 residues.

127. The method of claim 111, wherein the providing step comprises isolation of the one or more peptides from a natural source or comprises chemical synthesis of the peptide.

128. The method of claim 111, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide.

129. The method of claim 128, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide and at least one additional peptide, with a *proviso* that neither an additional peptide nor a combination of the peptide and an additional peptide comprise an entire native antigen.

130. The method of claim 111, wherein the first complexing step and/or the further complexing step occurs *in vitro*.

131. The method of claim 111, wherein the first complexing step and/or the further complexing step occurs *in vivo*.

132. The method of claim 111, wherein the first contacting step and/or the further contacting occurs *in vitro*.

133. The method of claim 111, wherein the first contacting step and/or the further contacting occurs *in vivo*.

134. The method of claim 92 comprising inducing an immune response, said method comprising steps of:

providing a nucleic acid encoding a peptide comprising a putative HLA epitope of about 8-11 amino acids in length, said epitope comprising an HLA-B27 structural supermotif associated with binding to multiple HLA molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of R, H, and K and a second amino acid anchor residue selected from the group consisting of F, Y, L, W, M, I, V, and A as the carboxyl-terminal amino acid residue of the epitope;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with a first HLA-B27 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with at least a further HLA-B27 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the further complex.

135. The method of claim 134, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-B*1401, HLA-B*1402, HLA-B*1509, HLA-B*2702, HLA-B*2703, HLA-B*2704, HLA-B*2705, HLA-B*2706, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*7301, HLA-B*2701, HLA-B*2707, HLA-B*2708, HLA-B*3802, HLA-B*3903, HLA-B*3904, HLA-B*3905, HLA-B*4801, HLA-B*4802, HLA-B*1510, HLA-B*1518, and HLA-B*1503 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

136. The method of claim 134, wherein the providing step comprises providing a nucleic acid encoding the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

137. The method of claim 134, wherein the providing step comprises providing a a nucleic acid encoding a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

138. The method of claim 92 comprising making an immunogenic peptide that bears an HLA-B58 supermotif and inducing an immune response, said peptide comprising an epitope consisting of about 8-11 residues that binds to multiple HLA molecules, and when bound to such an HLA molecule induces a cytotoxic T cell response, said method comprising steps of:

providing an amino acid sequence of an antigen of interest or a peptide fragment thereof, having an amino terminus and a carboxyl terminus;

identifying a putative T cell epitope within said amino acid sequence or a peptide fragment thereof, whereby said putative epitope comprises the structural HLA-B58 supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid residue at position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of A, T, and S, and a residue selected from the group consisting of F, W, Y, L, I, V, M, and A as a carboxyl-terminal amino acid residue of the epitope;

obtaining a peptide fragment derived from the antigen that comprises the HLA-B58 structural supermotif;

testing a complex of said peptide fragment and a first HLA-B58 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope;

testing a complex of said peptide fragment and at least a second HLA-B58 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope; and,

selecting said peptide fragment comprising the HLA-B58 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope when in complex with the first HLA-B58 supertype molecule and when in complex with the at least a second HLA-B58 supertype molecule.

139. The method of claim 138, wherein the peptide fragment has 8, 9, 10 or 11 residues.

140. The method of claim 138, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

141. The method of claim 138, further comprising a step of determining binding affinity of the peptide fragment for an HLA molecule selected from the group consisting of HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, and HLA-B*1517.

142. The method of claim 141, further comprising a step of identifying the peptide fragment which has a binding affinity of an IC_{50} of less than about 500 nM for the HLA molecule.

143. The method of claim 138, wherein the obtaining step comprises expressing in a cell a recombinant nucleic acid molecule that encodes the peptide fragment.

144. The method of claim 143, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the peptide fragment and one or more additional peptides; with a *proviso* that neither the peptide fragment, the one or more additional peptides, nor any combination of the peptide fragment and the one or more additional peptides comprise an entire native antigen.

145. The method of claim 138, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

146. The method of claim 138, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vitro*.

147. The method of claim 138, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vivo*.

148. The method of claim 138, wherein the providing step comprises providing an amino acid sequence of an antigen of interest which is a cancer-associated antigen.

149. The method of claim 148, wherein the providing step comprises providing an amino acid sequence from an antigen that is HER2/neu, p53, MAGE, or prostate antigen.

150. The method of claim 138, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

151. The method of claim 150, wherein the providing step comprises providing an amino acid sequence from an antigen that is an HBV, HCV, HIV, HPV, or malaria antigen.

152. The method of claim 92 comprising making an HLA-B58 supermotif peptide that binds to an HLA molecule at an IC_{50} of less than about 500 nM, the method comprising steps of:

(a) providing an amino acid sequence of an antigen of interest;

(b) identifying within said sequence a putative T cell epitope from the provided amino acid sequence, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-B58 structural supermotif associated with peptide binding to multiple HLA-B58 supertype molecules, said structural motif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of A, T, and S, and a second amino acid anchor residue selected from the group consisting of F, W, Y, L, I, V, M, and A as carboxyl-terminal amino acid residue of the epitope;

(c) obtaining a peptide fragment of said antigen of interest that comprises the HLA-B58 structural supermotif;

(d) contacting said peptide fragment of step (c) with a first HLA molecule selected from the group consisting of HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, and HLA-B*1517;

(e) contacting said peptide fragment of step (c) with a second HLA molecule selected from the group consisting of HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, and HLA-B*1517, wherein the second HLA molecule is different from the first HLA molecule;

(f) determining binding affinity of the peptide fragment for the first and the second HLA molecule; and,

(g) selecting the peptide fragment that comprise an HLA-B58 structural supermotif that binds to at least the first or at least the second HLA molecule at a binding affinity of an IC_{50} of less than about 500 nM.

153. The method of claim 152, further comprising a step of:

(h) contacting an HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, or HLA-B*1517 restricted cytotoxic T lymphocyte with a complex of the peptide of step (g) and an HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, or HLA-B*1517 molecule, respectively.

154. The method of claim 152, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vitro*.

155. The method of claim 152, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vivo*.

156. The method of claim 92 comprising inducing an immune response with a peptide comprising an epitope consisting of about 8-11 residues that will bind to multiple HLA-B58 supertype molecules and induce an HLA-restricted cytotoxic T cell response, said method comprising steps of:

providing a peptide comprising a putative T cell epitope, said putative epitope comprising an HLA-B58 structural supermotif associated with peptide binding to multiple HLA-B58 supertype molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of A, T, and S, and a

second amino acid anchor residue selected from the group consisting of F, W, Y, L, I, V, M, and A as the carboxyl-terminal amino acid residue of the epitope;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with a first HLA-B58 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with at least a further HLA-B58 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the at least a further complex.

157. The method of claim 156, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, and HLA-B*1517 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

158. The method of claim 156, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

159. The method of claim 158, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof which comprises an IC_{50} of less than about 50 nM for at least one HLA molecule.

160. The method of claim 158, wherein the at least one HLA molecule is selected from the group consisting of: HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, and HLA-B*1517 molecules.

161. The method of claim 156, wherein the providing step comprises providing the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

162. The method of claim 156, wherein said provided peptide is connected to another molecule to create a compound, with a *proviso* that neither said peptide, said another molecule, nor said compound comprise an entire native antigen.

163. The method of claim 162, wherein the another molecule is a lipid.

164. The method of claim 162, wherein the another molecule is a T helper epitope.

165. The method of claim 162, wherein the another molecule is a cytotoxic T lymphocyte (CTL) epitope.

166. The method of claim 165, wherein the another molecule is the peptide.

167. The method of claim 162, wherein the another molecule is a carrier molecule.

168. The method of claim 156, wherein the providing step comprises providing a peptide from a cancer-associated antigen.

169. The method of claim 156, wherein the providing step comprises providing a peptide from an antigen that is derived from a pathogenic agent.

170. The method of claim 156, wherein the providing step comprises providing a peptide of more than 11 residues.

171. The method of claim 156, wherein the providing step comprises providing a peptide of 8, 9, 10 or 11 residues.

172. The method of claim 156, wherein the providing step comprises isolation of the one or more peptides from a natural source or comprises chemical synthesis of the peptide.

173. The method of claim 156, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide.

174. The method of claim 173, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide and at least one additional peptide, with a *proviso* that neither an additional peptide nor a combination of the peptide and an additional peptide comprise an entire native antigen.

175. The method of claim 156, wherein the first complexing step and/or the further complexing step occurs *in vitro*.

176. The method of claim 156, wherein the first complexing step and/or the further complexing step occurs *in vivo*.

177. The method of claim 156, wherein the first contacting step and/or the further contacting occurs *in vitro*.

178. The method of claim 156, wherein the first contacting step and/or the further contacting occurs *in vivo*.

179. The method of claim 92 comprising inducing an immune response, said method comprising steps of:

providing a nucleic acid encoding a peptide comprising a putative HLA epitope of about 8-11 amino acids in length, said epitope comprising an HLA-B58 structural supermotif associated with binding to multiple HLA molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of A, T, and S and a second amino acid anchor residue selected from the group consisting of F, W, Y, L, I, V, M, and A as the carboxyl-terminal amino acid residue of the epitope;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with a first HLA-B58 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with at least a further HLA-B58 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the further complex.

180. The method of claim 179, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-B*5701, HLA-B*5702, HLA-B*5801, HLA-B*5802, HLA-B*1516, and HLA-B*1517 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

181. The method of claim 179, wherein the providing step comprises providing a nucleic acid encoding the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

182. The method of claim 179, wherein the providing step comprises providing a a nucleic acid encoding a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

183. The method of claim 92 comprising making an immunogenic peptide that bears an HLA-B62 supermotif and inducing an immune response, said peptide comprising an epitope consisting of about 8-11 residues that binds to multiple HLA molecules, and when bound to such an HLA molecule induces a cytotoxic T cell response, said method comprising steps of:

providing an amino acid sequence of an antigen of interest or a peptide fragment thereof, having an amino terminus and a carboxyl terminus;

identifying a putative T cell epitope within said amino acid sequence or a peptide fragment thereof, whereby said putative epitope comprises the structural HLA-B62 supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid residue at position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of Q,

L, I, V, M, and P, and a residue selected from the group consisting of F, W, Y, L, I, V, M, and A as a carboxyl-terminal amino acid residue of the epitope;

obtaining a peptide fragment derived from the antigen that comprises the HLA-B62 structural supermotif;

testing a complex of said peptide fragment and a first HLA-B62 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope;

testing a complex of said peptide fragment and at least a second HLA-B62 supertype molecule for an ability to be recognized by cytotoxic T cells and to thereby induce a cytotoxic T cell response to the epitope; and,

selecting said peptide fragment comprising the HLA-B62 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope when in complex with the first HLA-B62 supertype molecule and when in complex with the at least a second HLA-B62 supertype molecule.

184. The method of claim 183, wherein the peptide fragment has 8, 9, 10 or 11 residues.

185. The method of claim 183, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

186. The method of claim 183, further comprising a step of determining binding affinity of the peptide fragment for an HLA molecule selected from the group consisting of HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, and HLA-B*1510.

187. The method of claim 186, further comprising a step of identifying the peptide fragment which has a binding affinity of an IC_{50} of less than about 500 nM for the HLA molecule.

188. The method of claim 183, wherein the obtaining step comprises expressing in a cell a recombinant nucleic acid molecule that encodes the peptide fragment.

189. The method of claim 188, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the peptide fragment and one or more additional peptides; with a *proviso* that neither the peptide fragment, the one or more additional peptides, nor any combination of the peptide fragment and the one or more additional peptides comprise an entire native antigen.

190. The method of claim 183, wherein the obtaining step obtains the peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

191. The method of claim 183, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vitro*.

192. The method of claim 183, wherein the testing step with the first HLA molecule and/or the testing step with the second HLA molecule occurs *in vivo*.

193. The method of claim 183, wherein the providing step comprises providing an amino acid sequence of an antigen of interest which is a cancer-associated antigen.

194. The method of claim 193, wherein the providing step comprises providing an amino acid sequence from an antigen that is HER2/neu, p53, MAGE, or prostate antigen.

195. The method of claim 183, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

196. The method of claim 195, wherein the providing step comprises providing an amino acid sequence from an antigen that is an HBV, HCV, HIV, HPV, or malaria antigen.

197. The method of claim 92 comprising making an HLA-B62 supermotif peptide that binds to an HLA molecule at an IC_{50} of less than about 500 nM, the method comprising steps of:

(a) providing an amino acid sequence of an antigen of interest;

(b) identifying within said sequence a putative T cell epitope from the provided amino acid sequence, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-B62 structural supermotif associated with peptide binding to multiple HLA-B62 supertype molecules, said structural motif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of Q, L, I, V, M, and P, and a second amino acid anchor residue selected from the group consisting of F, W, Y, L, I, V, M, and A as carboxyl-terminal amino acid residue of the epitope;

(c) obtaining a peptide fragment of said antigen of interest that comprises the HLA-B62 structural supermotif;

(d) contacting said peptide fragment of step (c) with a first HLA molecule selected from the group consisting of HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, and HLA-B*1510;

(e) contacting said peptide fragment of step (c) with a second HLA molecule selected from the group consisting of HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, and HLA-B*1510, wherein the second HLA molecule is different from the first HLA molecule;

(f) determining binding affinity of the peptide fragment for the first and the second HLA molecule; and,

(g) selecting the peptide fragment that comprise an HLA-B62 structural supermotif that binds to at least the first or at least the second HLA molecule at a binding affinity of an IC_{50} of less than about 500 nM.

198. The method of claim 197, further comprising a step of:

(h) contacting an HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, or HLA-B*1510 restricted cytotoxic T lymphocyte with a complex of the peptide of step (g) and an HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, or HLA-B*1510 molecule, respectively.

199. The method of claim 197, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vitro*.

200. The method of claim 197, wherein the contacting step of step (d) and/or the contacting step of step (e) occurs *in vivo*.

201. The method of claim 92 comprising inducing an immune response with a peptide comprising an epitope consisting of about 8-11 residues that will bind to multiple HLA-B62 supertype molecules and induce an HLA-restricted cytotoxic T cell response, said method comprising steps of:

providing a peptide comprising a putative T cell epitope, said putative epitope comprising an HLA-B62 structural supermotif associated with peptide binding to multiple HLA-B62 supertype molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of Q, L, I, V, M, and P, and a second amino acid anchor residue selected from the group consisting of F, W, Y, L, I, V, M, and A as the carboxyl-terminal amino acid residue of the epitope;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with a first HLA-B62 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the provided peptide, or a fragment thereof which comprises the supermotif, with at least a further HLA-B62 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the at least a further complex.

202. The method of claim 201, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, and HLA-B*1510 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

203. The method of claim 201, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.

204. The method of claim 203, wherein the providing step comprises providing a peptide or supermotif-comprising fragment thereof which comprises an IC_{50} of less than about 50 nM for at least one HLA molecule.

205. The method of claim 203, wherein the at least one HLA molecule is selected from the group consisting of: HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, and HLA-B*1510 molecules.

206. The method of claim 201, wherein the providing step comprises providing the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

207. The method of claim 201, wherein said provided peptide is connected to another molecule to create a compound, with a *proviso* that neither said peptide, said another molecule, nor said compound comprise an entire native antigen.

208. The method of claim 207, wherein the another molecule is a lipid.

209. The method of claim 207, wherein the another molecule is a T helper epitope.

210. The method of claim 207, wherein the another molecule is a cytotoxic T lymphocyte (CTL) epitope.

211. The method of claim 210, wherein the another molecule is the peptide.

212. The method of claim 207, wherein the another molecule is a carrier molecule.

213. The method of claim 201, wherein the providing step comprises providing a peptide from a cancer-associated antigen.

214. The method of claim 201, wherein the providing step comprises providing a peptide from an antigen that is derived from a pathogenic agent.

215. The method of claim 201, wherein the providing step comprises providing a peptide of more than 11 residues.

216. The method of claim 201, wherein the providing step comprises providing a peptide of 8, 9, 10 or 11 residues.

217. The method of claim 201, wherein the providing step comprises isolation of the one or more peptides from a natural source or comprises chemical synthesis of the peptide.

218. The method of claim 201, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide.

219. The method of claim 218, wherein the providing step comprises expressing a recombinant nucleic acid molecule that encodes the peptide and at least one additional peptide, with a *proviso* that neither an additional peptide nor a combination of the peptide and an additional peptide comprise an entire native antigen.

220. The method of claim 201, wherein the first complexing step and/or the further complexing step occurs *in vitro*.

221. The method of claim 201, wherein the first complexing step and/or the further complexing step occurs *in vivo*.

222. The method of claim 201, wherein the first contacting step and/or the further contacting occurs *in vitro*.

223. The method of claim 201, wherein the first contacting step and/or the further contacting occurs *in vivo*.

224. The method of claim 92 comprising inducing an immune response, said method comprising steps of:

providing a nucleic acid encoding a peptide comprising a putative HLA epitope of about 8-11 amino acids in length, said epitope comprising an HLA-B62 structural supermotif associated with binding to multiple HLA molecules, said structural supermotif comprising a first amino acid anchor residue at a position two from the epitope's amino-terminal amino acid residue, said first anchor residue selected from the group consisting of Qj, L, I, V, M, and P and a second amino acid anchor residue selected from the group consisting of F, W, Y, L, I, V, M, and A as the carboxyl-terminal amino acid residue of the epitope;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with a first HLA-B62 supertype molecule, whereby a first complex is prepared;

contacting a cytotoxic T lymphocyte (CTL) with the first complex, whereby a CTL response is induced to the first complex;

complexing the peptide encoded by the provided nucleic acid, or a fragment thereof which comprises the supermotif, with at least a further HLA-B62 supertype molecule, whereby at least a further complex is prepared; and,

contacting a cytotoxic T lymphocyte (CTL) with the further complex, whereby a CTL response is induced to the further complex.

225. The method of claim 224, wherein the first HLA molecule and the further HLA molecule are selected from the group consisting of HLA-B*1501, HLA-B*1502, HLA-B*1513, HLA-B*5201, HLA-B*1301, HLA-B*1302, HLA-B*1504, HLA-B*1505, HLA-B*1506, HLA-B*1507, HLA-B*1515, HLA-B*1520, HLA-B*1521, HLA-B*1512, HLA-B*1514, and HLA-B*1510 molecules, and wherein the further HLA molecule is different from the first HLA molecule.

226. The method of claim 224, wherein the providing step comprises providing a nucleic acid encoding the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

227. The method of claim 224, wherein the providing step comprises providing a a nucleic acid encoding a peptide or supermotif-comprising fragment thereof that comprises an IC_{50} of less than about 500 nM for at least one HLA molecule.